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Report of the interlaboratory comparison organised by the EU Reference Laboratory for Food Contact Materials

ILC02 2009- Bisphenol A in 50% aqueous ethanol (milk simulant)

Laboratory performance and precision criteria of a harmonised method

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Report of the interlaboratory comparison

Bisphenol A in 50% aqueous ethanol

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1. Summary

The Institute for Health and Consumer Protection (IHCP) of the European Commission's Directorate-General Joint Research Centre hosts the Community Reference Laboratory for Food Contact Materials (EURL-FCM). One of its core tasks is to organize interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs).

This report presents the results of the second ILC exercise of the EURL-FCM for the year 2009, which focused on the determination of Bisphenol A (BPA) in 50% aqueous ethanol as a food simulant for milk. The general aim was to develop and perform the validation of a method for the analysis of Bisphenol A as model substance for a polycarbonate (PC), since it is a material that has typically been used as baby bottles and therefore typically in contact with mostly milk-type products. The strategy rose from the implementation of the new milk simulant 50% Ethanol (EtOH) in Commission Directive 2007/19/EC that current CEN standards for specific migration have not addressed yet.

The first specific intention of the exercise on BPA was to validate an extension of scope of EN13130 Part 13 to be extended to include this new simulant 50% EtOH, i.e. the method for the quantification of BPA in 50% EtOH in the range around the legislative specific migration limit (SML, 0.6 mg/kg). In addition, rather than just validating the method at levels close to the SML as required for compliance purposes, it was agreed that a second validation range would also be studied to allow validation data to be generated for exposure purposes.

The test materials used in this exercise were spiked samples prepared by EURL-FCM with several level of Bisphenol A (Sigma Aldrich) in 50% ethanol.

In total four 50% ethanol solutions containing different concentrations of Bisphenol A were provided for analysis encompassing concentrations of relevance to exposure determination and compliance determination. The homogeneity and stability studies were performed by the EURL-FCM laboratory. Standard operating procedures (SOPs) for the two approaches were also written. The spiked samples and SOP(s) were sent in August and the deadline for the submission of results was mid-October. Participation of local laboratories under NRLs was encouraged (by producing 60 samples). There were 31 participants from twenty-five countries to whom samples were dispatched and 26 of which submitted results. From the EURL-NRL network 18 laboratories out of 24 reported results. There were 3 guests from Spain and 3 from Germany that provided results as well. Participants were invited to report four replicates measurements in repeatability conditions. This was done by most of the participants. The ILC was closed permanently in the end of October for statistical interpretation. The results of analyses were received and statistically interpreted. The assigned values were obtained as a consensus values after applying the robust statistics to the results obtained from the participants. Laboratory results were rated with z-scores in accordance with ISO 13528 [1]. Standard deviations for ILC comparison (also called target standard deviations) were set based on Horwitz equation and Horrat ratio 0.5. The results and preliminary report were discussed in the plenary of December 2009. The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results. Absolute minimum of participating laboratories for conducting a precision experiment is 8. Some of the NRLs communicated the lack of the

possibility to follow exactly the method for BPA determination as they used different analytical technique (e.g. LC-MS instead of HPLC/FLD). Since the aim of the ILC was directed towards method validation there was a communication to the participants that different techniques were not fit to the scope of this ILC. That was the reason for slightly lower percentage of reported test results as compared with the other ILC exercise of 2009. As a conclusion of the precision exercise on the quantification of Bisphenol A in the new milk simulant 50% ethanol, this ILC showed that:
The participation in the ILC was satisfactory for the purpose of the study towards validation with 24 laboratories.
The validation of the method based on HPLC-FLD according to the description based mostly on the previous CEN standard TS 13130-13 was successful with more than 8 valid results thanks to the proactive involvement of the NRLs.
The precision that can be suggested were of
15% reproducibility SD and 6% repeatability SD for the 0.0067 mg/kg level,
10% reproducibility SD and 4% repeatability SD for the 0.0.21 mg/kg level,
6% reproducibility SD and 2% repeatability SD for the 0.0.75 mg/kg level,
6% reproducibility SD and 0.2% repeatability SD for the 0.56 mg/kg level.

With respect to the scarcity of data previously available in the validation performed as reported in the CEN standard TS 13130-13 (issued version of 2005), this validation also provides a great breadth of valuable detailed and traceable raw data, which should prove extremely relevant for the creation of an extension of the standard from CEN.

2. Introduction

ILC studies are an essential and very important element of laboratory quality assurance, which allows individual laboratories to compare their analytical results with those from other laboratories while providing them objective standards to perform against.

It is one of the core duties of the Community Reference Laboratories to organise interlaboratory comparisons, as is stated in Regulation (EC) No 882/2004 of the European Parliament and of the Council [6].

In accordance with the above requirements the Community Reference Laboratory for Food Contact Material (EURL-FCM) organised in 2009 for the second year interlaboratory comparison tests for the network of appointed National Reference Laboratories (NRLs).

The scopes of the interlaboratory comparison (ILC) tests for 2009 were discussed and agreed in the plenary meeting with all NRLs held in December 2008 at JRC, Ispra, Italy. On that meeting the preference was expressed a work item on the determination of Bisphenol A in 50% aqueous ethanol simulant as second ILC (ILC02). It was agreed that this ILC would be directed towards method validation.

2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A, $C_{15}H_{16}O_2$, PM/Ref. 13480) is a monomer used in the manufacture of certain plastics materials as polycarbonate and articles intended to come into contact with foodstuffs. After manufacture residual

Bisphenol A can remain in the finished product (e.g. polycarbonate bottles) and may migrate/release into foodstuffs coming into contact with that product (e.g milk or milk formula as in baby bottles).

prEN 13130-13:2006 was prepared by Technical Committee CEN/TC 194 "Utensils in contact with food". The document was prepared by Subcommittee SC 1 of TC 194 as one of a series of analytical test methods for plastics materials and articles in contact with foodstuffs and submitted to the CEN Enquiry. This document will supersede CEN/TS 13130-13:2005.

prEN 13130-13:2006 aim to decrease the minimum level for quantitative determination of Bisphenol A by using fluorescence (FLD) detection instead of DAD (diod-array detector) as in version from 2005. The pre-validated version however concerns only 3% acetic acid and 10% ethanol as aqueous food simulants. As 50% ethanol:water is introduced as a new food simulant for milk (infant formulae) for baby bottles, one of the scopes of the present ILC is to provide reliable validation data for 50% aqueous ethanol as a food simulant.

As agreed on the EURL-NRL plenary from 2-5 July 2009 the ILC included as well a low calibration range with the aim to be used for exposure assessment purposes for the real release/migration at low concentration level of BPA from material in contact with foodstuff due to the potential concerns even at lower exposure levels.

It was decided that aim of this ILC and hence the validation would be the two concentration ranges:

- the calibration range, applicable to the determination of Bisphenol A at concentrations between 0,030 mg and 1,2 mg of Bisphenol A per litre of food simulant according to pr EN 13130 : 2006 for compliance assessment

- the calibration range, applicable to the determination of Bisphenol A at concentrations between 0.002 mg and 0.030 mg of Bisphenol A per litre of food simulant for exposure assessment purposes

3. Scope

The scope of this ILC was directed towards two objectives:

- to validate preliminary agreed upon analytical method drafted as SOP for determination of BPA in 50% aqueous ethanol;

- to test the competence of the appointed NRLs to analyse BPA in the new food simulant for milk;

The concentration levels were chosen in both ranges – high level in accordance with the existing legislation [3, 4] for enforcement purposes and in low level for exposure assessment.

The assessment of the measurement results was undertaken on the basis of requirements laid down in international standards and guidelines [1, 2, 9, 10]

4. Time frame

EURL-FCM drafted a proposal for SOP based on the prEN 13130-13:2006. As agreed on the EURL-NRL plenary from 2-5 July 2009, the only difference was the inclusion of the low calibration range with the aim to be used for the real low concentration levels of BPA migration necessary for exposure assessment.

The samples were prepared at the end of July 2009

Invitation letters were sent to the laboratories on 13 July 2009 (Annex 2). Laboratories were invited to fill in a letter of confirmation of their participation (Annex 3)

The samples were dispatched to participants on 18 August 2009, together with a letter accompanying the samples (Annex 4), the Standard Operating Procedure of the analytical method to be used for the exercise (Annex 1), detailed instructions for compilation of the results in electronic format (Annex 6), a format for the compilation of results to be eventually sent in non-electronic format (Annex 7) and electronic files where the result should be inserted.

The participants were asked to fill in a letter of confirmation of the receipt of the samples (Annex 5)

The deadline for reporting was set to 12 October 2009.

5. Test material

Samples of 50% aqueous ethanol spiked at different levels of BPA were prepared by the EURL-FCM as presented at table 1

Exercise	Name	Sample
ILC 02 2009	**BPA1**	1 screw cap vial of 50% ethanol (30ml) spiked at level 1
	BPA2	1 screw cap vial of 50% ethanol (30ml) spiked at level 2
	BPA3	1 screw cap vial of 50% ethanol (30ml) spiked at level 3
	BPA4	1 screw cap vial of 50% ethanol (30ml) spiked at level 4

Table1. Samples for ILC02

5.1 Preparation

Preparation and homogenisation of the test material was done by the EURL-FCM laboratory according to the procedure described in Annex 9.

After spiking and homogenization the solvent was dispensed in screw cap glass vials of approximately 40 ml capacity.

5.2 Homogeneity assessment

The samples were tested for homogeneity by the EURL Laboratory.

Ten randomly selected test specimens for each sample (BPA01, BPA02, BPA03 and BPA04) were analysed in duplicate for bisphenol A.

Homogeneity was evaluated by the Prolab Software according to IUPAC International Harmonised Protocol [10] and to the method proposed in the ISO 13528 [1]. The results together with their statistical evaluation are given in Annex 10.

All test materials has shown sufficient homogeneity for the target standard deviation shown in table 8.

5.3 Stability test

Randomly selected specimens for each sample (BPA1, BPA2, BPA3 and BPA4) were stored at 4 different temperature conditions (-18°C, +4ºC, room temperature, +40ºC). The test samples were monitored for stability by the EURL laboratory, by mean of BPA determination, from the date of preparation to the data of closing the ILC02 - 12 October 2009. The samples were analysed in duplicate every 3 weeks over the given time frame.

Stability was evaluated as described in ISO GUIDE 35:2006 [16].

The evaluation of data was carried out by performing a linear regression on the determined concentrations of BPA (mean values) vs time. For a stable material it is expected that the intercept is (within uncertainty) equal to the assigned value, whereas the slope does not differ significantly from zero.

Being the linear regression equation:

$$Y_{(\text{BPA conc, mg/kg})} = b_0 + b_1 X_{(\text{time, weeks})}$$

the slope is not significantly different from zero if the following requirement is respected;

$$|b_1| < t_{0,95,n-2} \cdot s(b_1)$$

where

b1	is the slope obtained from the linear regression,
$t_{0.95,n-2}$	is the Student's t-factor for n-2 degrees of freedom and p = 0.95 (95% level of confidence) and
$s(b_1)$	is the uncertainty associated with the slope.

This can be calculated as follows:

$$s(b_1) = \frac{s}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}}$$

The value of s (standard deviation of the time-points) can be obtained from:

$$s^2 = \frac{\sum_{i=1}^{n}(Y_i - b_0 - b_1 X_i)^2}{n-2}$$

where n is the number of points of the linear regression.

The results together with their statistical evaluation are given in Annex 12

It should be pointed out that no significant trend was observed for the test samples at all temperature conditions (-18C, -4C, RT and +40C) for the time of the ILC. It could be concluded that the environment conditions of sample storage did not influence the stability of the samples within two months after their preparation.

5.4 Distribution

The samples were dispatched to the participants by the EURL-FCM on 18 August 2009. Each participant received:

a) a box containing the test materials;

b) an accompanying letter with instructions on sample handling (Annex 4)

c) instructions to the participant for reporting (Annex 6);

d) a form that had to be sent back after receipt of the sample to confirm its arrival (cf. Annex 5) and

e) a form for reporting the result in non-electronic format (Annex 7)

6. Instructions to participants

Concrete instructions were given to all participants in a letter that accompanied the samples (Annex 4).

Laboratories were asked to perform four replicate measurements in repeatability conditions and report them. Participants were asked to follow the distributed SOP as close as possible and eventual deviation to be reported. The results were to be reported using the unit of measure indicated in the instruction letter.

The results were to be reported in a special ProLab [5] software form as presented at table 2

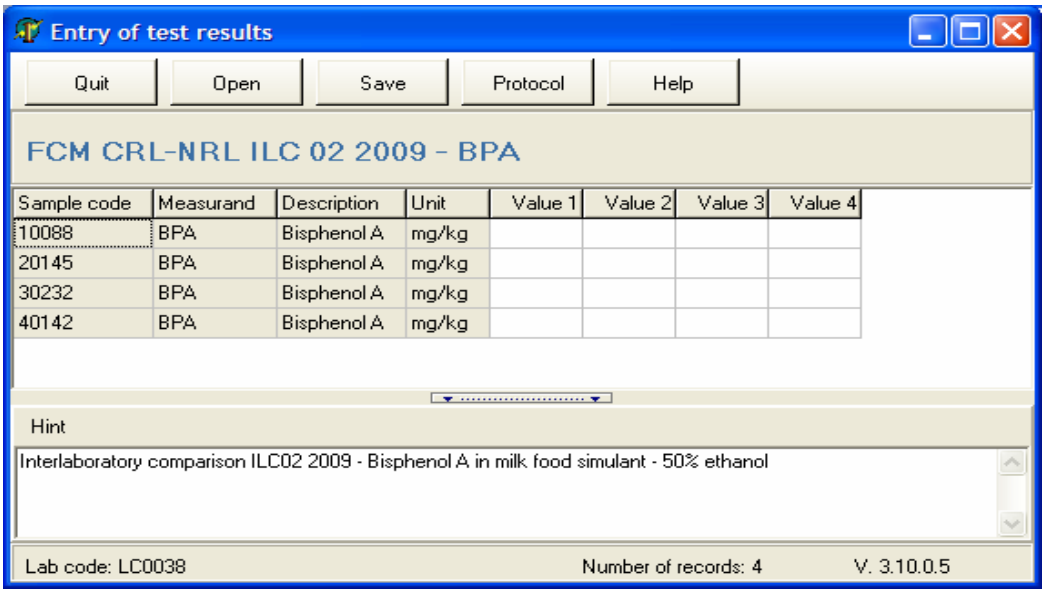


Table 2 ProLab software form for reporting of the result

7. Approaches for statistical evaluation of results

7.1. Evaluation of the BPA method performance characteristics – methods for determination of the consensus value and repeatability (r) and reproducibility (R) standard deviation

Statistical evaluation of the results was performed using the ProLab software [5] applying different algorithms for the determination of the consensus value and repeatability (r) and reproducibility (R) standard deviation.

ISO 5725-2 [14] is an approach for the statistical analysis of method validation interlaboratory studies, i.e. it should not be used for the analysis of proficiency testing schemes. For the calculations according to those standards the following specific assumptions are made:

- all laboratories (apart from only a very few outlier laboratories) must have equal analytical performance in order to guarantee that distribution of the test results is close to the normal distribution (*clearly this assumption cannot be made for PT's);

- all laboratories must use the same analytical method;

- the method requires replicates.

ISO 5725-2 applies the Grubbs test for the outlier identification of individual test results and laboratory mean values. Additionally tests for the identification of exceeding intra laboratory standard deviations are applied (Cochran test and F-test,

respectively). It is a common experience when analysing data from precision experiments to find data that are on the borderline between stragglers and outliers, so that the judgments may have to be made that affect the results of the calculation. This may be unsatisfactory. Applying robust methods as it is described in ISO 5725-5 [15] allows the data to be analysed in such a way that it is not require to make decision that affect the results of the calculations. The algorithm of ISO 5725-5 (Algorithm A +S) is similar to one in ISO 13528 [1]. In case those conditions assumed normally for method validation ILC hold and the results are normally distributed, classical statistics in ISO 5725-2 give results that are very similar to robust statistics in 5275-5 or Q/Hampel algorithm in DIN 38402 A45 [7] and ISO/TS 20612 [8].

7.2. Identification of modes using kernel density plotting

Kernel density plots were additionally used to identify multi modality in the reported values' distributions.

Frequently analytical results from a collaborative study are not normally distributed or contain values from different populations giving rise to multiple distribution modes. These modes can be visualised by using Kernel density plots [12, 13]. In case the results are not normally distributed the classical statistics from ISO 5725-2 should not be applied

Kernel density plots are computed by the ProLab software [5] from the analytical results by representing the individual numeric values each as a normalised Gaussian distribution centered on the respective analytical value. The sum of these normal distributions forms then the Kernel density distribution. There is a proposal for using a KDM mode as an estimation of the assigned value of one ILC.

7.3. Mandel's h- and k-statistics

Mandel's h-statistic and Mandel's k-statistic [11] present measures for graphically surveying the consistency of the data. They are helpful for method and laboratory assessment. For answering the questions if there are differences between the mean values of the laboratories, Mandel's h-statistic could be considered. In order to assess the variance of each laboratory compared to the variances of the other laboratories, Mandel's k-statistic is useful. Mandel's h- and k- values are calculated by ProLab software following ISO 5725.

The examination of the plots of Mandel's h- and k-statistics may indicate that specific laboratories exhibit patterns of results that are markedly different from the others. This is indicated by (compared to the other laboratories) consistently high or low variation and/or extreme (high or low) mean values.

Various patterns can appear in the plot of Mandel's h-statistic. All laboratories can have both positive and negative values. Individual laboratories may tend to give either all positive or all negative values. This is no unusual pattern, but it may suggest that a common source of laboratory bias exists.

If one laboratory stands out on the k-statistic as having many large values, the respective laboratory has a poorer repeatability precision than the other laboratories. A laboratory could give rise to consistently small k-values because of such factors as excessive rounding of its data or an insensitive measurement scale.

7.4. Evaluation criteria for laboratory performance – type of z-scores

Individual laboratory performance was expressed in terms of z and z'-scores in accordance with ISO 13528[1] and the International Harmonised Protocol[10]

$$z = \frac{(x_{lab} - X_{assigned})}{\sigma_p}$$

$$z' = \frac{(x_{lab} - X)}{\sqrt{\sigma_p{}^2 + u^2_{assigned}}}$$

where

x_{lab}	is the measurement result reported by a participant
$X_{assigned}$	is the assigned value
σ_p	is the target standard deviation for proficiency assessment
$u_{assigned}$	is the standard uncertainty of the assigned value

The z- and z'-scores can be interpreted as follow:

$\|z\| \leq 2$	satisfactory result
$2 < \|z\| \leq 3$	questionable result
$\|z\| > 3$	unsatisfactory result

The z-scores compared the participant's deviation from the assigned value with target standard deviation accepted for the interlaboratory comparison σ_p

z'-scores could be used when the assigned value is not calculated using the results reported by the participants. z'-score takes in consideration the uncertainty of the assigned values. In case the guidelines for limiting the uncertainty of the assigned value $u_{assigned} < 0.3\ \sigma_p$ [1] are met, then z'-scores would be similar to z'-scores Present ILC02 uses the mean values of all results reported by the participant as an assigned value. Therefore the z'-scores could not be used for assessment of the laboratory performance.

For results reported as "smaller than" (<-values), the reported value was not used in any calculations and no evaluation of the measurement results was made. No scores were given.

7.5. Youden Plot

Youden plots are a graphical technique for analyzing ILC data when each laboratory has run test samples in duplicate or for at least 2 identical sample/measurand combinations. It is a simple but effective method for comparing both the within-laboratory variability and the between-laboratory variability.

8. Comments on results and conclusions

8.1 General observations

There were thirty-one participants from twenty-five countries to whom samples are dispatched. They all received the samples. The ILC was closed permanently in the end of October for statistical interpretation.

Twenty-six laboratories submitted results. From the EURL-NRL network 20 laboratories out of 26 reported results. There were 3 guests from Spain and 3 from Germany that provided results as well. As requested, most of the laboratories reported four replicate results under repeatability conditions.

The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results. Absolute minimum of participating laboratory for conducting a precision experiment is 8. Some of the NRLs communicated the lack of the possibility to follow exactly the method for BPA determination as they use different analytical technique (LC-MS instead of HPLC/FLD). Since the aim of the ILC02 was directed towards method validation there was a communication to the participants that different techniques were not fit to the scope of this ILC. That was the reason for slightly lower percent of reported test results as compared with ILC01.

8.2 Method performance characteristics from the precision experiment

Summary of the mean values and reproducibility and repeatability standard deviation calculated according to 3 different algorithms – classical ISO 5725-2, robust ISO 5725-5 and Hampel algorithm (ISO 20612:2007 and DIN 38402 A45) by ProLab software - as well as the reference values are given in Table 3.

It should be mentioned that the robust mean derived from the results coincided well with the reference values calculated based on formulation (table 3), in spite of the stability problem for the high concentration level in the last week before the deadline for the ILC02. The difference between $x_{mean} - X_{ref}$ was less then twice its standard uncertainty for all levels.

$(\frac{(1{,}23s^*)^2}{p} + u_X{}^2)^{1/2}$ where

u_x is the uncertainty of the reference values;
s* is the robust standard deviation;

p is the number of participating laboratories

Figure 1 represents the correlation between the concentration level and the corresponding repeatability/reproducibility standard deviations

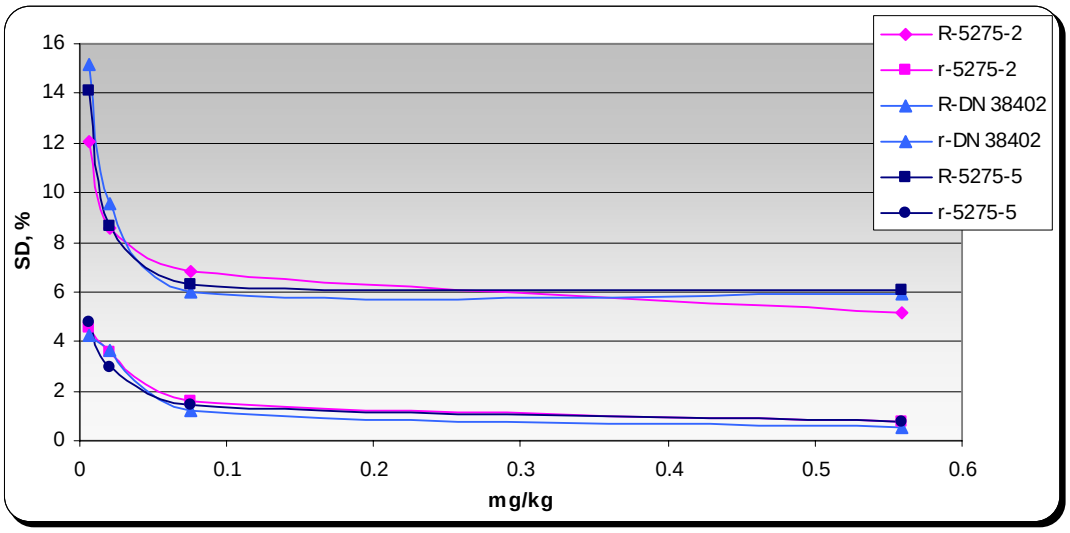


Figure 1 – Correlation between reproducibility/repeatability SD and concentration level in the validated concentration ranges.

In classical ISO 5725-2 algorithm the Grubs and Cochran test showed few outliers and strugglers. The outliers were taken away from further statistical elaboration performed with that method. Their number and type per samples are shown at table 4

	Grubs outliers (mean value)	Cochran outliers (standard deviation)	Grubs + Cochran outliers
BPA01		**4**	
BPA02		**1**	
BPA03		**1**	**2**
BPA04	**1**	**2**	

Table 4. Number of Grubs and Cochran outliers for all the samples

ISO 5275-2 classical

Sample	Mean	Ref.value	Rel.Reprod. S.D.	Reprod.S.D.	Rel.Repeat. S.D.	Repeat.S.D.	Assigned value	Ref.-Mean values	SD difference	
	mg/kg	mg/kg	%	mg/kg	%	mg/kg	mg/kg			
BPA01	0.0067	0.0069	11.09	0.0008	4.52	0.00030	0.0067	0.0002	0.0016	TRUE
BPA02	0.0204	0.0213	7.82	0.0017	3.56	0.00073	0.0204	0.0009	0.0035	TRUE
BPA03	0.0753	0.0775	8.26	0.0051	2.03	0.0012	0.0753	0.0022	0.0102	TRUE
BPA04	0.565	0.581	4.87	0.292	0.75	0.0042	0.565	0.0158	0.583	TRUE

ISO 5275-5 robust

Sample	Mean	Ref.value	Rel.Reprod. S.D.	Reprod.S.D.	Rel.Repeat. S.D.	Repeat.S.D.	Assigned value	Ref.-Mean values	SD difference	
	mg/kg	mg/kg	%	mg/kg	%	mg/kg	mg/kg			
BPA01	0.0067	0.0069	14.12	0.0010	4.81	0.00032	0.0067	0.0002	0.0019	TRUE
BPA02	0.0205	0.0213	8.20	0.0018	2.93	0.00060	0.0205	0.0008	0.0035	TRUE
BPA03	0.075	0.0775	5.48	0.0047	1.46	0.0011	0.075	0.0025	0.0095	TRUE
BPA04	0.560	0.581	5.78	0.339	0.73	0.0041	0.560	0.0213	0.6784	TRUE

DIN 38402 robust Hample estimator

Sample	Mean	Ref.value	Rel.Reprod. S.D.	Reprod.S.D.	Rel.Repeat. S.D.	Repeat.S.D.	Assigned value	Ref.-Mean values	SD difference	
	mg/kg	mg/kg	%	mg/kg	%	mg/kg	mg/kg			
BPA01	0.0067	0.0069	15.51	0.0010	4.1	0.00027	0.0067	0.0002	0.0021	TRUE
BPA02	0.0205	0.0213	9.76	0.0020	3.37	0.0007	0.0205	0.0008	0.0040	TRUE
BPA03	0.0753	0.0775	6.6	0.005	1.22	0.0009	0.0753	0.0022	0.0099	TRUE
BPA04	0.562	0.581	6.13	0.344	0.49	0.0027	0.562	0.0194	0.6885	TRUE

Table 3. Mean values and repeatability/reproducibility SD calculated by 3 different algorithms

In the robust algorithm (ISO 5725-5 and DIN 38402 A45) all the value are taken in the calculations.

It should be pointed out that the calculated mean values according to the three different algorithms corresponded very well. Slight but not significant differences could be observed in estimation of the repeatability/reproducibility standard deviations.

As a result of the precision experiment conducted with ILC02 BPA in 50% aqueous ethanol the precision parameters shown in Table 5 could be suggested for the method:

Concentration level mg/kg	Reproducibility (R), %	Horrat R	Repeatability (r), %
0.0067	**15**	**0.7**	**5**
0.021	**10**	**0.4**	**4**
0.075	**7**	**0.3**	**1.5**
0.56	**6**	**0.3**	**0.8**

Table 5. Summary of the results from the precision experiment for validation of the method

Horrat (R) (**HOR**witz**RAT**io) values are calculated for all the concentration levels according to the formulae:

Horrat (R) = Reprod. SD (%)/ Predicted truncated Horwitz SD(%)

Horrat value is now one of the acceptability criteria for many of the recently adopted chemical methods of analysis of AOAC INTERNATIONAL, the European Union, and other European organisations dealing with food analysis (e.g., European Committee for Standardisation and Nordic Analytical Committee) [17]. Consistent deviations from the ratio on the low side (values <0.5) may indicate unreported averaging or excellent training and experience; consistent deviations on the high side (values >2) may indicate inhomogeneity of the test samples, need for further method optimisation or training, operating below the limit of determination, or an unsatisfactory method.

In spite of the fact that Horwitz formulae (resp. Horrat values) should not be dependent on any analyte or matrix, a very simple analytical procedure requiring only instrumental analysis without any sample preparation could be expected to have lower values for reproducibility SD and respectively Horrat values. This is the case with the procedure for BPA determination in 50% ethanol which requires only direct injection in the HPLC system and therefore results in low Horrat values (0.7-0.3).

8.3 Laboratory results and scores

For calculation of the z-score, hence the performance of each laboratory the most important decision to be taken by the organiser is the assigned value and the target standard deviation against which the performance will be assessed.

For the ***assigned values*** in ILC02 a robust mean was chosen as a consensus assigned value. In spite the fact that the robust mean and the reference values calculated based on formulation are significantly not different there is a significant trend for degradation observed as a result of the stability tests in the last weeks especially for the samples in the upper concentration range (annex 11). As some of the laboratories reported their results around or a little bit after the deadline (12 October) the slightly lower mean robust values could be explained based on that rationale. That is why a robust mean is regarded as more representative for the assigned value then the reference values calculated from the formulation.

For the ***target standard deviation*** in most of the cases Horwitz SD is a good compromise. When the concentrations are very low however the Horwitz SD is too high and a truncated Horwitz SD is used. In case of very simple method, analysis of analyte in a solvent as it is the case in present ILC, a more restrictive target SD could be used, e.g. Horrat=0.5. Empirical Horwitz can be calculated when more then 2-3 concentration levels are included in the ILC in the exactly same matrix.

The results as reported by the participants were summarised in Table 6 (a-d).

Three sets of figures were provided for each of the four samples in Fig 2 (1-4). Each set included (a) individual laboratories values and their mean and standard deviation, (b) the Kernel Density plot, (c) the z- scores. Red bars indicate the Grubs (B- mean value) and Cochran (C – standard deviation) outliers according to ISO 5725-2

In Figure 3 Mandel's h- and Mandel's k-statistics are shown as calculated according to ISO 5725-2. Values differing statistically significant from values of the other laboratories are marked in a different colour: a red bar indicates a value significant to the significance level of 1% while a yellow bar indicates a value significant to the level of 5%. The outcome of the Mandel's h- and Mandel's k-statistics presented in table 7 were similar to and in correspondence with the Grubs and Cochran outliers' tests according to ISO 5725-2.

Number of laboratories with Mandel h- statistics non consistent with 5% and 1% significance level	
5%	1%
3	**4**
Number of laboratories with Mandel k- statistics non consistent with 5% and 1% significance level	
5%	1%
3	**6**

Table 7. Summary of the number of laboratories outliers according to the Mandel tests

Table 6.a: Laboratories' raw test results, their mean values and corresponding z-score

FCM CRL-NRL ILC 02 2009 - BPA

Test results

Sample:	**BPA01**	**Assigned value:**	**0.0066 mg/kg (Empirical value)**
Measurand:	**Bisphenol A**	**Rel. repeatability s.d.:**	**4.82%**
Method:	**ISO 5725-5 (Alg. A+S)**	**Rel. reproducibility s.d.:**	**14.12%**
No. of laboratories:	**23**	**Limits of tolerance:**	**0.0048 - 0.0085 mg/kg (|Z-Score**

Laboratory code	M 1	M 2	M 3	M 4	M	S.d.
LC0000	0.0067	0.0067	0.0059	0.0065	0.0065	0.0004
LC0003	0.0060	0.0069	0.0067	0.0072	0.0067	0.0005
LC0004	0.0060	0.0090	0.0040	0.0100	0.0073	0.0028
LC0005	0.0056	0.0053	0.0050	0.0053	0.0053	0.0003
LC0006	0.0064	0.0064	0.0062	0.0064	0.0064	0.0001
LC0010	0.0061	0.0063	0.0064	0.0065	0.0063	0.0002
LC0011	0.0056				0.0056	
LC0013	0.0059	0.0059	0.0061	0.0059	0.0060	0.0001
LC0014	<0.1300	<0.1300	<0.1300	<0.1300		
LC0016						
LC0017	0.0115	0.0110	0.0074	0.0068	0.0092	0.0024
LC0018	0.0076	0.0075	0.0081	0.0080	0.0078	0.0003
LC0020						
LC0021	0.0070	0.0060	0.0060	0.0060	0.0063	0.0005
LC0025	<0.0100	<0.0100	<0.0100	<0.0100		
LC0026	0.0090	0.0080	0.0070	0.0090	0.0083	0.0010
LC0028						
LC0029						
LC0031	0.0064	0.0063	0.0066	0.0066	0.0065	0.0002
LC0037						
LC0038	0.0065	0.0065	0.0064	0.0068	0.0066	0.0002
LC0040	0.0058	0.0058	0.0055	0.0060	0.0058	0.0002
LC0041	0.0080	0.0074	0.0073	0.0072	0.0075	0.0004
LC0042	0.0068	0.0073	0.0071	0.0071	0.0071	0.0002
LC0043						
LC0044	0.0052	0.0052	0.0055	0.0051	0.0052	0.0002
LC0048	0.0087	0.0074	0.0083	0.0065	0.0077	0.0010
LC0049	0.0068	0.0067	0.0066	0.0065	0.0067	0.0001
LC0050						
LC0054	0.0065	0.0066	0.0062	0.0063	0.0064	0.0002
LC0055	0.0060	0.0061	0.0060	0.0061	0.0060	0.0001
LC0056	0.0065	0.0065	0.0075	0.0075	0.0070	0.0006

Table 6.b: Laboratories' raw test results, their mean values and corresponding z-score

FCM CRL-NRL ILC 02 2009 - BPA

Test results

Sample:	**BPA02**	**Assigned value:**	**0.0204 mg/kg (Empirical value)**
Measurand:	**Bisphenol A**	**Rel. repeatability s.d.:**	**2.93%**
Method:	**ISO 5725-5 (Alg. A+S)**	**Rel. reproducibility s.d.:**	**8.20%**
No. of laboratories:	**25**	**Limits of tolerance:**	**0.0171 - 0.0237 mg/kg (|Z-Score**

Laboratory code	M 1	M 2	M 3	M 4	M	S.d.
LC0000	0.019	0.019	0.019	0.019	0.019	0.000
LC0003	0.020	0.021	0.022	0.022	0.021	0.001
LC0004	0.019	0.020	0.021	0.017	0.019	0.002
LC0005	0.017	0.017	0.017	0.016	0.017	0.000
LC0006	0.020	0.021	0.021	0.021	0.021	0.000
LC0010	0.021	0.021	0.021	0.021	0.021	0.000
LC0011	0.017				0.017	
LC0013	0.021	0.021	0.021	0.021	0.021	0.000
LC0014	<0.1300	<0.1300	<0.1300	<0.1300		
LC0016						
LC0017	0.024	0.027	0.020	0.023	0.024	0.003
LC0018	0.023	0.022	0.022	0.023	0.022	0.000
LC0020						
LC0021	0.019	0.020	0.019	0.018	0.019	0.001
LC0025	0.020	0.021	0.020	0.022	0.021	0.001
LC0026	0.023	0.022	0.021	0.022	0.022	0.001
LC0028						
LC0029						
LC0031	0.020	0.020	0.019	0.019	0.020	0.001
LC0037	0.019	0.019	0.019	0.018	0.019	0.000
LC0038	0.021	0.021	0.021	0.021	0.021	0.000
LC0040	0.020	0.021	0.020	0.021	0.021	0.000
LC0041	0.022	0.022	0.022	0.022	0.022	0.000
LC0042	0.019	0.019	0.019	0.019	0.019	0.000
LC0043						
LC0044	0.019	0.019	0.018	0.018	0.019	0.001
LC0048	0.021	0.020	0.022	0.022	0.021	0.001
LC0049	0.020	0.020	0.020	0.020	0.020	0.000
LC0050						
LC0054	0.022	0.022	0.022	0.022	0.022	0.000
LC0055	0.020	0.020	0.021	0.022	0.021	0.001
LC0056	0.020	0.020	0.021	0.024	0.021	0.002

Table 6.c: Laboratories' raw test results, their mean values and corresponding z-score

FCM CRL-NRL ILC 02 2009 - BPA

Test results

Sample:	**BPA03**	**Assigned value:**	**0.0748 mg/kg (Empirical value)**
Measurand:	**Bisphenol A**	**Rel. repeatability s.d.:**	**1.45%**
Method:	**ISO 5725-5 (Alg. A+S)**	**Rel. reproducibility s.d.:**	**5.48%**
No. of laboratories:	**25**	**Limits of tolerance:**	**0.0666 - 0.0830 mg/kg (|Z-Score**

Laboratory code	M 1	M 2	M 3	M 4	M	S.d.
LC0000	0.081	0.079	0.078	0.079	0.079	0.001
LC0003	0.071	0.073	0.072	0.073	0.072	0.001
LC0004	0.089	0.087	0.089	0.094	0.090	0.003
LC0005	0.073	0.073	0.074	0.074	0.073	0.000
LC0006	0.071	0.072	0.070	0.071	0.071	0.001
LC0010	0.075	0.076	0.077	0.078	0.076	0.001
LC0011	0.067				0.067	
LC0013	0.079	0.078	0.078	0.079	0.079	0.000
LC0014	<0.1300	<0.1300	<0.1300	<0.1300		
LC0016						
LC0017	0.077	0.078	0.073	0.074	0.076	0.002
LC0018	0.054	0.053	0.058	0.060	0.056	0.003
LC0020						
LC0021	0.076	0.078	0.075	0.076	0.076	0.001
LC0025	0.075	0.077	0.076	0.074	0.076	0.001
LC0026	0.069	0.076	0.076	0.075	0.074	0.003
LC0028						
LC0029						
LC0031	0.070	0.070	0.070	0.070	0.070	0.000
LC0037	0.069	0.069	0.068	0.069	0.069	0.001
LC0038	0.077	0.077	0.077	0.077	0.077	0.000
LC0040	0.072	0.071	0.072	0.071	0.071	0.000
LC0041	0.078	0.076	0.078	0.079	0.078	0.001
LC0042	0.075	0.075	0.074	0.075	0.075	0.000
LC0043						
LC0044	0.075	0.075	0.075	0.076	0.075	0.000
LC0048	0.077	0.077	0.074	0.076	0.076	0.001
LC0049	0.084	0.076	0.075	0.076	0.077	0.004
LC0050						
LC0054	0.083	0.083	0.085	0.083	0.084	0.001
LC0055	0.076	0.077	0.077	0.076	0.076	0.001
LC0056	0.075	0.073	0.076	0.075	0.075	0.001

Table 6.d: Laboratories' raw test results, their mean values and corresponding z-score

FCM CRL-NRL ILC 02 2009 - BPA

Test results

Sample:	**BPA04**	**Assigned value:**	**0.5592 mg/kg (Empirical value)**
Measurand:	**Bisphenol A**	**Rel. repeatability s.d.:**	**0.73%**
Method:	**ISO 5725-5 (Alg. A+S)**	**Rel. reproducibility s.d.:**	**5.78%**
No. of laboratories:	**26**	**Limits of tolerance:**	**0.4945 - 0.6238 mg/kg (|Z-Score**

Laboratory code	M 1	M 2	M 3	M 4	M	S.d.
LC0000	0.586	0.586	0.587	0.587	0.586	0.000
LC0003	0.524	0.526	0.527	0.529	0.527	0.002
LC0004	0.540	0.539	0.537	0.544	0.540	0.003
LC0005	0.510	0.509	0.510	0.508	0.509	0.001
LC0006	0.528	0.527	0.530	0.528	0.528	0.001
LC0010	0.580	0.580	0.591	0.582	0.583	0.006
LC0011	0.521				0.521	
LC0013	0.590	0.591	0.590	0.590	0.590	0.000
LC0014	0.420	0.411	0.413	0.429	0.418	0.008
LC0016						
LC0017	0.603	0.602	0.600	0.598	0.601	0.002
LC0018	0.552	0.588	0.511	0.528	0.545	0.033
LC0020						
LC0021	0.590	0.593	0.595	0.599	0.594	0.004
LC0025	0.580	0.580	0.570	0.560	0.573	0.010
LC0026	0.514	0.526	0.520	0.527	0.522	0.006
LC0028						
LC0029						
LC0031	0.595	0.589	0.589	0.587	0.590	0.003
LC0037	0.553	0.553	0.554	0.549	0.552	0.002
LC0038	0.568	0.569	0.567	0.569	0.568	0.001
LC0040	0.545	0.543	0.539	0.548	0.544	0.004
LC0041	0.593	0.600	0.602	0.611	0.601	0.007
LC0042	0.565	0.566	0.565	0.566	0.565	0.001
LC0043						
LC0044	0.580	0.580	0.580	0.570	0.577	0.005
LC0048	0.568	0.576	0.582	0.575	0.575	0.006
LC0049	0.551	0.549	0.550	0.550	0.550	0.001
LC0050						
LC0054	0.557	0.529	0.537	0.536	0.540	0.012
LC0055	0.578	0.580	0.580	0.580	0.580	0.001
LC0056	0.560	0.560	0.573	0.560	0.564	0.007

Figure 2a: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z'-scores (c)

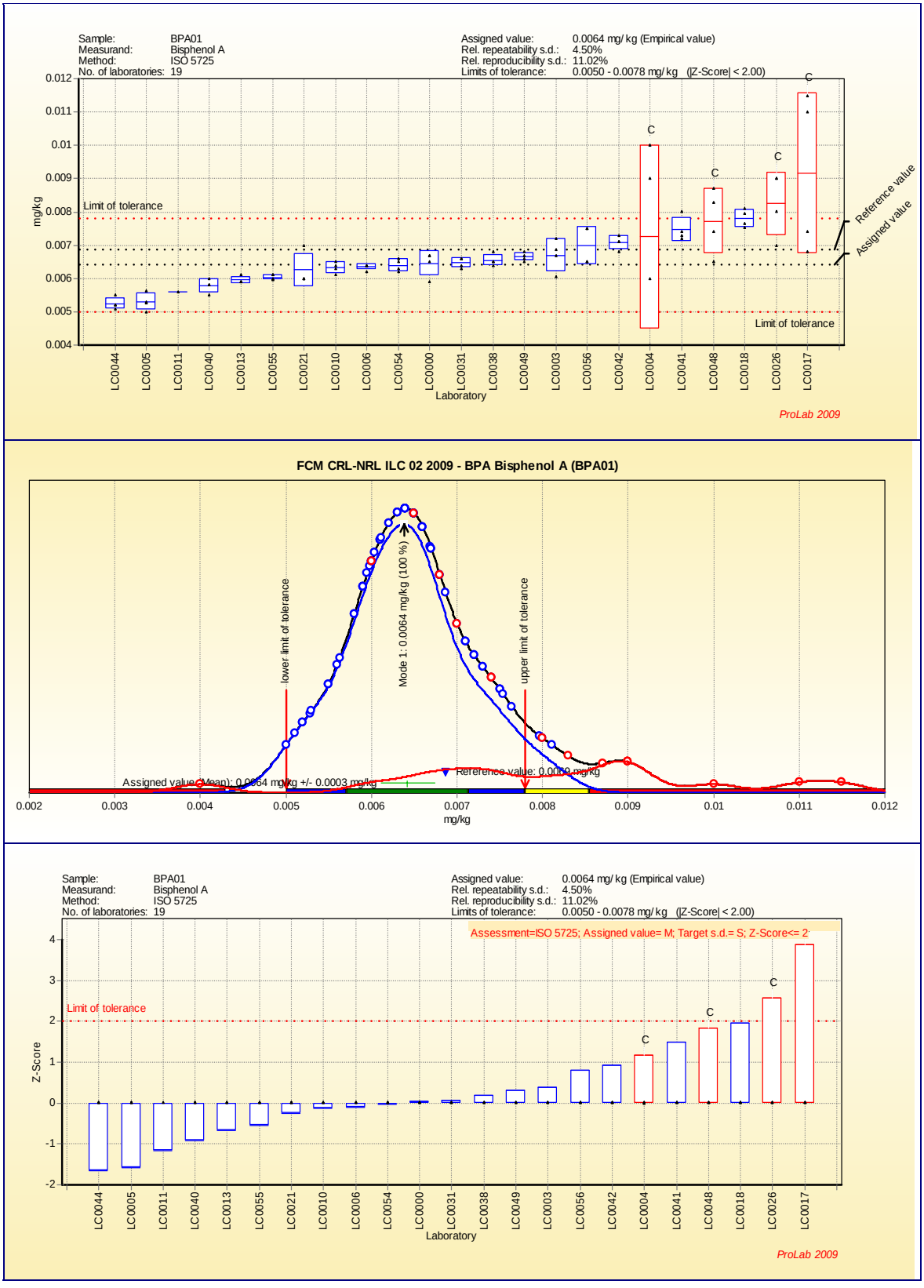


Figure 2b: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z'-scores (c)

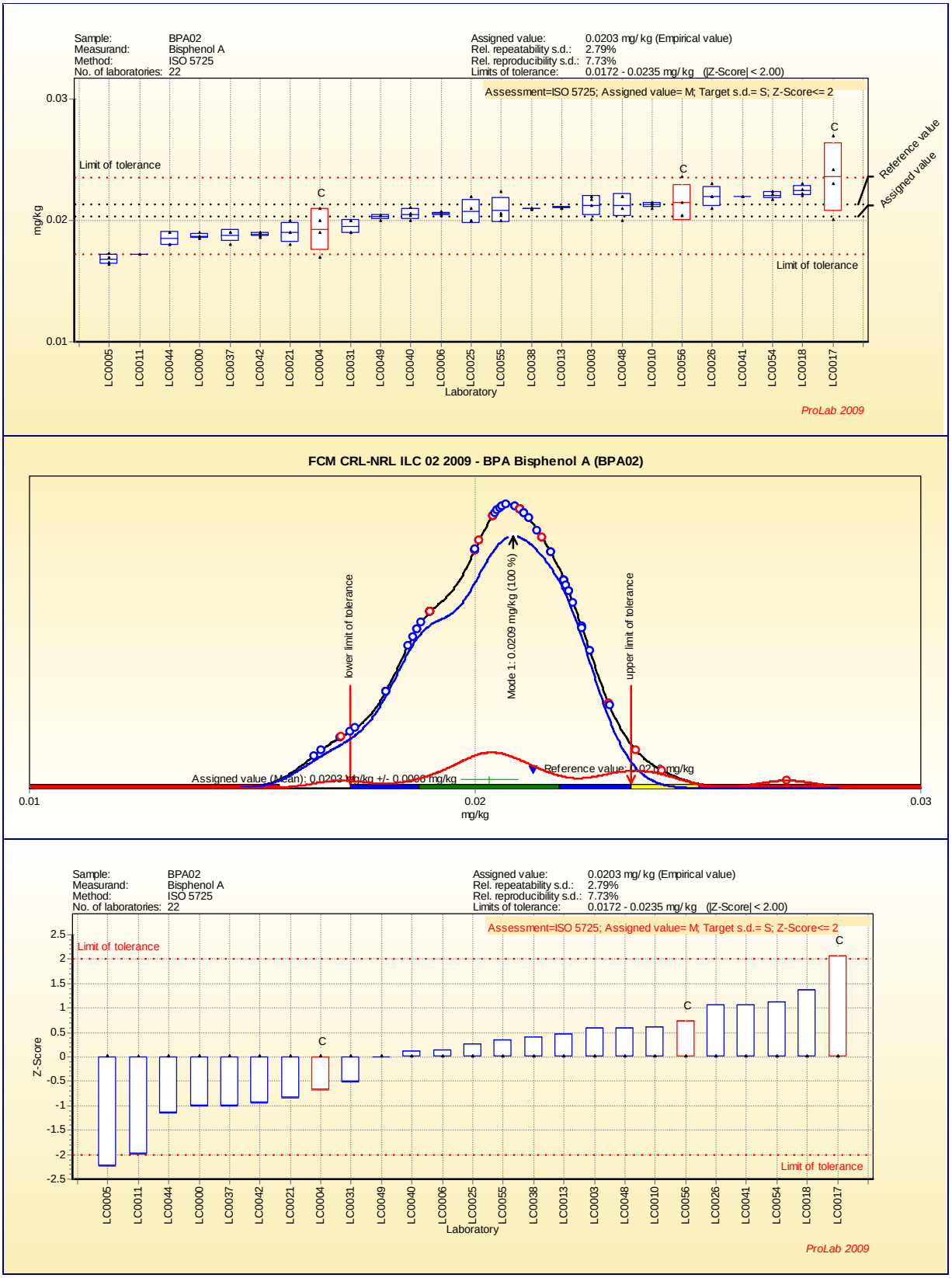


Figure 2c: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z'-scores (c)

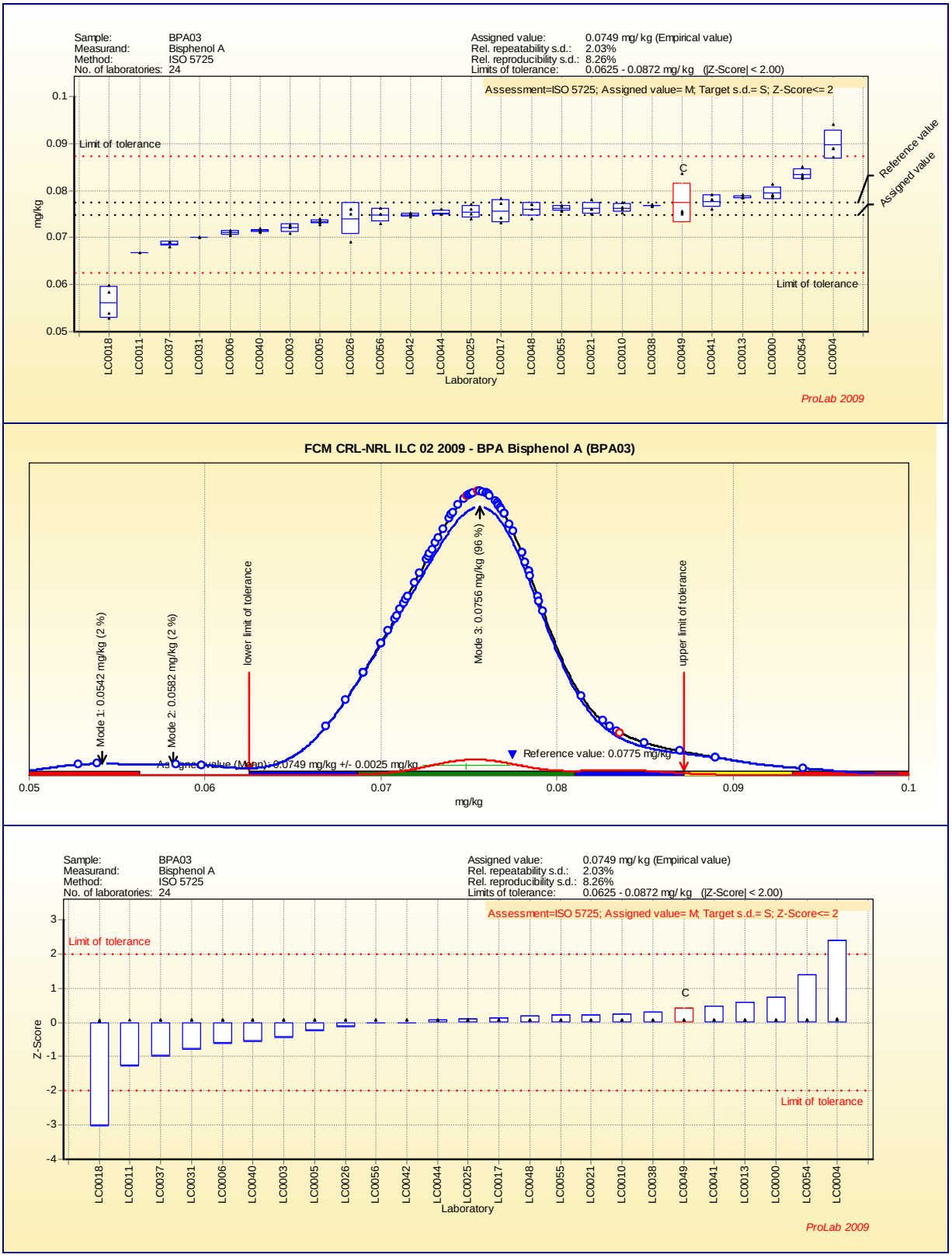


Figure 2d: Summary graphs of the laboratory's test results with their repeatability SD (a), Kernel Density plot (b) and z-scores (c)

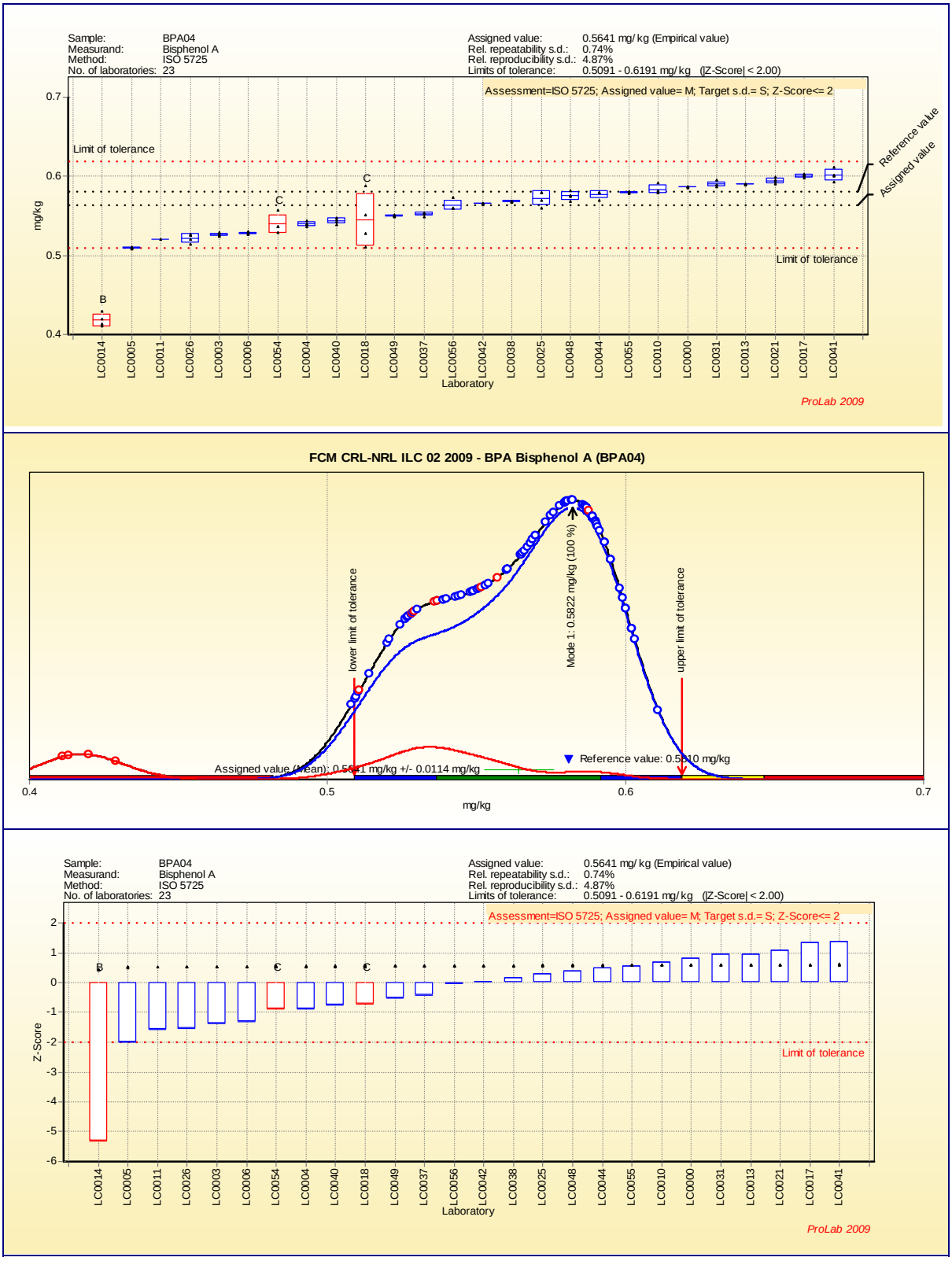
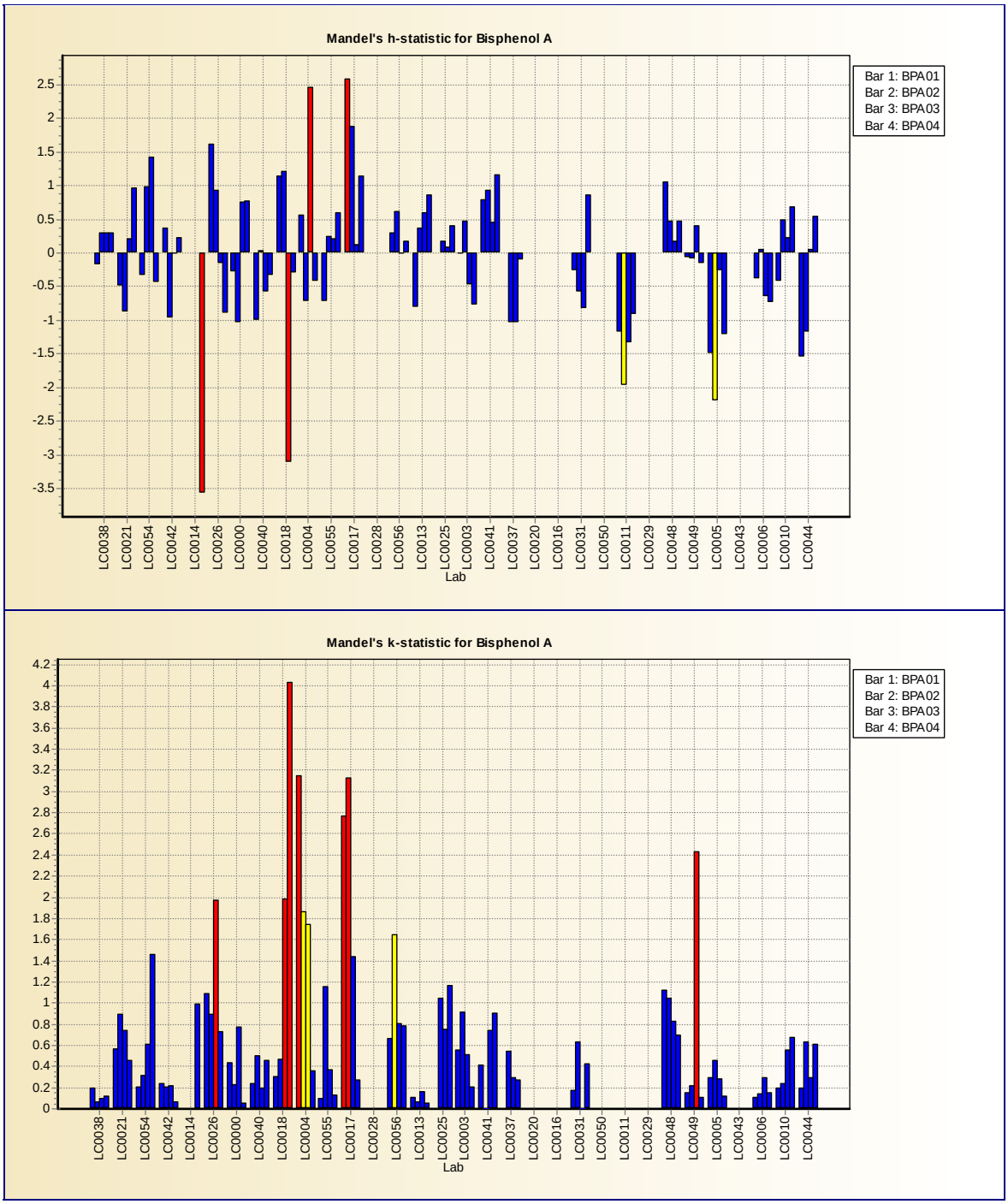


Figure 3:. Mandel h- and k-statistics for BPA in 50% aqueous ethanol



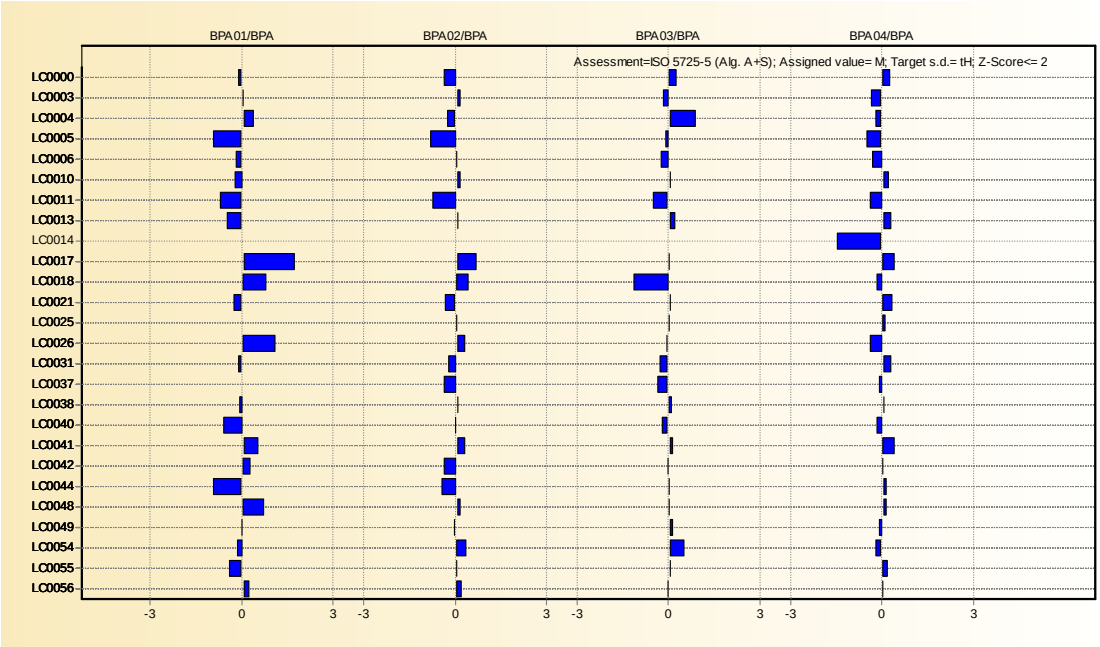
* for 1% significant level the indicative Mandel's h value is 2.43 and k-value (for n=4 replicates) is 1.90. Laboratories with higher values are marked in red

* for 5% significant level the indicative Mandel's h value is 1.90 and k-value (for n=4 replicates) is 1.60. Laboratories with higher values are marked in yellow

** The legend next to the figure explains the sequence of the bars for each laboratory, i.e. the first entry in the legend coincides with the bar at the farthest-left (for one laboratory), while the last legend entry coincides with the bar on the farthest-right (for one laboratory).

Figure 4. Summary of z -scores against different target SD

a) target SD = truncated Horwitz



b) target SD at Horrat = 0.5

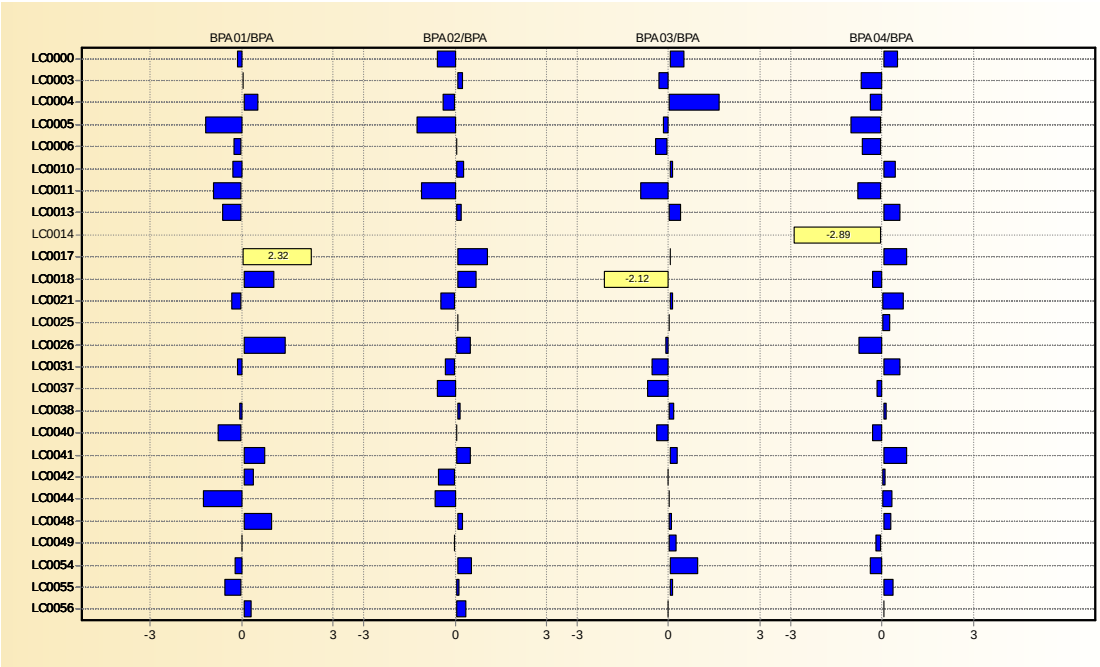
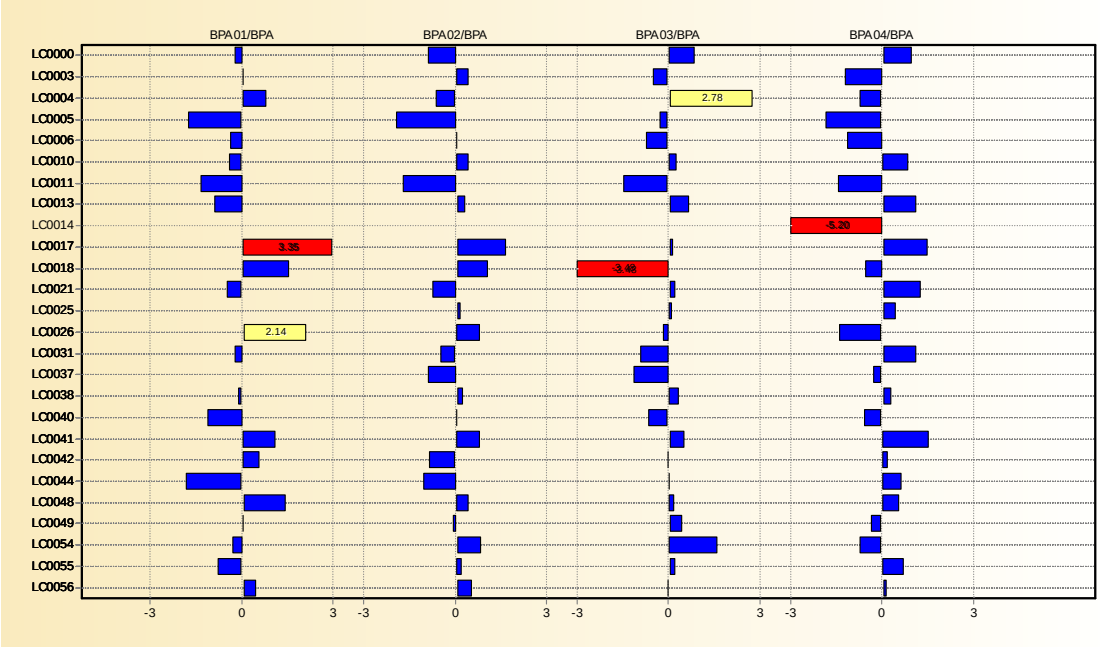


Figure 4. Summary of z -scores against different target SD

c) target SD =Emperical Horwitz



d) target SD = reproducibility SD of the ILC02 (ISO 5275-5 robust)

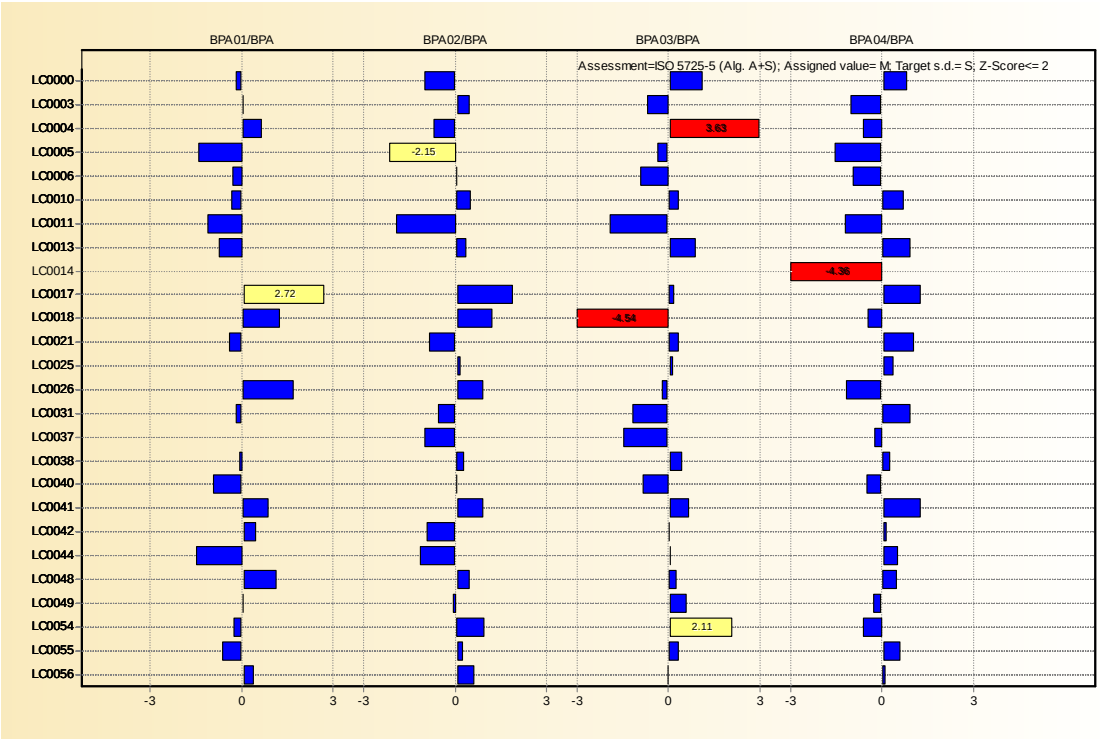


Table 8: Summary of z -scores against different target SD

	Truncated Horwitz				0.5 * HORWITZ				Empirical Horwitz				ILC02 reprod. SD (ISO 5275-5 robust)			
Laboratory	BPA01	BPA02	BPA03	BPA04	BPA01	BPA02	BPA03	BPA04	BPA01	BPA02	BPA03	BPA04	BPA01	BPA02	BPA03	BPA04
	22	22	22	17.5	16.6	14.2	11.7	8.7	11.5	9.2	7.2	4.8	14.12	8.2	5.48	5.78
LC0000	-0.12	-0.37	0.28	0.28	-0.16	-0.58	0.53	0.56	-0.23	-0.89	0.87	1	-0.19	-1	1.13	0.84
LC0003	0.05	0.19	-0.17	-0.33	0.07	0.29	-0.31	-0.67	0.1	0.45	-0.52	-1.2	0.08	0.51	-0.67	-1.01
LC0004	0.43	-0.26	0.91	-0.2	0.57	-0.4	1.69	-0.39	0.82	-0.61	2.78	-0.71	0.67	-0.69	3.63	-0.59
LC0005	-0.91	-0.8	-0.09	-0.51	-1.2	-1.24	-0.17	-1.02	-1.74	-1.91	-0.28	-1.84	-1.41	-2.15	-0.36	-1.54
LC0006	-0.19	0.03	-0.23	-0.32	-0.25	0.05	-0.43	-0.63	-0.36	0.08	-0.71	-1.14	-0.3	0.09	-0.92	-0.96
LC0010	-0.21	0.19	0.09	0.25	-0.27	0.3	0.17	0.49	-0.4	0.46	0.28	0.89	-0.32	0.52	0.36	0.75
LC0011	-0.7	-0.71	-0.48	-0.39	-0.93	-1.1	-0.9	-0.78	-1.35	-1.7	-1.48	-1.41	-1.1	-1.92	-1.93	-1.18
LC0013	-0.46	0.14	0.23	0.32	-0.62	0.22	0.42	0.64	-0.89	0.34	0.7	1.15	-0.72	0.39	0.91	0.96
LC0014				-1.44				-2.89				-5.2				-4.36
LC0017	1.75	0.71	0.05	0.43	2.32	1.09	0.1	0.85	3.35	1.69	0.16	1.53	2.72	1.9	0.2	1.29
LC0018	0.81	0.46	-1.13	-0.15	1.08	0.71	-2.12	-0.3	1.56	1.09	-3.48	-0.54	1.27	1.23	-4.54	-0.45
LC0021	-0.26	-0.31	0.09	0.36	-0.34	-0.48	0.16	0.72	-0.5	-0.75	0.26	1.29	-0.4	-0.84	0.34	1.09
LC0025		0.08	0.04	0.14		0.12	0.08	0.27		0.18	0.12	0.49		0.21	0.16	0.41
LC0026	1.11	0.36	-0.05	-0.38	1.48	0.55	-0.1	-0.77	2.14	0.85	-0.16	-1.38	1.73	0.95	-0.2	-1.16
LC0031	-0.1	-0.2	-0.29	0.32	-0.14	-0.31	-0.55	0.63	-0.2	-0.48	-0.9	1.14	-0.16	-0.54	-1.18	0.95
LC0037		-0.37	-0.37	-0.07		-0.57	-0.69	-0.14		-0.88	-1.14	-0.25		-0.99	-1.48	-0.21
LC0038	-0.05	0.12	0.12	0.09	-0.07	0.19	0.22	0.19	-0.1	0.29	0.36	0.34	-0.08	0.33	0.47	0.28
LC0040	-0.58	0.03	-0.21	-0.16	-0.77	0.04	-0.38	-0.32	-1.12	0.06	-0.63	-0.57	-0.91	0.07	-0.83	-0.48
LC0041	0.58	0.36	0.18	0.43	0.77	0.55	0.33	0.87	1.12	0.85	0.54	1.56	0.91	0.95	0.71	1.31
LC0042	0.31	-0.35	0	0.06	0.41	-0.54	0	0.13	0.59	-0.83	0	0.23	0.48	-0.93	0	0.19
LC0044	-0.94	-0.42	0.03	0.19	-1.25	-0.66	0.05	0.38	-1.81	-1.01	0.08	0.68	-1.47	-1.14	0.1	0.57
LC0048	0.75	0.19	0.07	0.16	1	0.29	0.13	0.33	1.45	0.45	0.22	0.59	1.17	0.51	0.28	0.5
LC0049	0.02	-0.02	0.16	-0.09	0.02	-0.04	0.29	-0.19	0.03	-0.05	0.48	-0.33	0.02	-0.06	0.62	-0.28
LC0054	-0.16	0.37	0.53	-0.2	-0.21	0.58	0.98	-0.4	-0.3	0.89	1.62	-0.72	-0.24	1	2.11	-0.6
LC0055	-0.41	0.1	0.09	0.21	-0.54	0.15	0.16	0.42	-0.78	0.24	0.26	0.76	-0.63	0.27	0.34	0.63
LC0056	0.26	0.24	0	0.05	0.34	0.37	0	0.09	0.49	0.57	0	0.16	0.4	0.64	0	0.14

Z-scores assessed the laboratory performance against some target standard deviation. As in the recent ILC02 the assigned value is calculated using the results reported by the participants z'-scores could not be used.

Since the present ILC02 was directed mainly towards method validation then assessment of the laboratory performance in Figure 4 and Table 8 are presented z-scores calculated against 4 different target SD

a) target SD = 0.5*Hotwitz (Horrat = 0.5)
b) target SD = Emperical Horwitz
c) target SD = truncated Horwitz
d) target SD = reproducibility SD of the ILC02

Taking into consideration that Horwitz formulae is derived for real matrix samples where at least some sample preparation step is necessary and that the matrix in the present ILC02 samples is only ethanol/water with a method which requires only direct injection into HPLC, the suggested target SD for laboratory assessment is at least 0.5*Horwitz. It can be seen that the real reproducibility standard deviation from this ILC02 is even much lower then 0.5*Horwitz.

Figure 5 represents the tolerance limits in relation to the concentration level of BPA in food simulant calculated against both target SD – 0.5*Horwitz and relative reproducibility SD of the ILC02. Tolerance limits are calculated at z-score < 2. Both graphs shows a trend of decreasing the range for tolerance limits with increasing the concentration, with stronger decrease in the second one, where they are calculated on the bases of real relative SD of the ILC02.

The Youden plot displays a combined graphic of the results of one measurand at two different concentration levels. Fig.6 a is for the low concentration range and Fig. 6 b is for the high concentration range. Such a presentation allows identifying systematic effects in the laboratory-specific deviations for both matrixes. It gives an immediate idea of the dominating sources of error in the results. Laboratories having results in the upper left or lower right hand corner of the diagram have analyses dominated by random error. On the other hand, laboratories having results close to the 45° line shown in the plot, but far away from the assigned value have results dominated by systematic error.

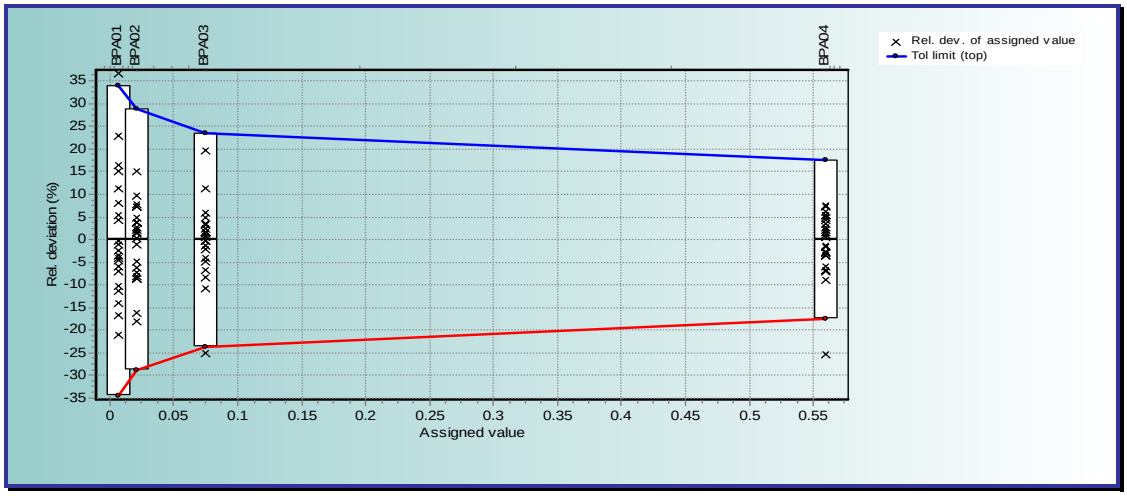
Youden plots presented on Figure 6 show slight correlation between the results in the low concentration range (correlation coefficient is 0.62). For the higher concentration range no correlation could be observed.

Figure 7 represents the laboratory mean values against its repeatability SD for all concentration levels in acetonitrile and oil. Tolerance limits shown on the graphs were calculated based on classical Horwitz SD. The figure illustrates a clear picture of the results outside the tolerance limits.

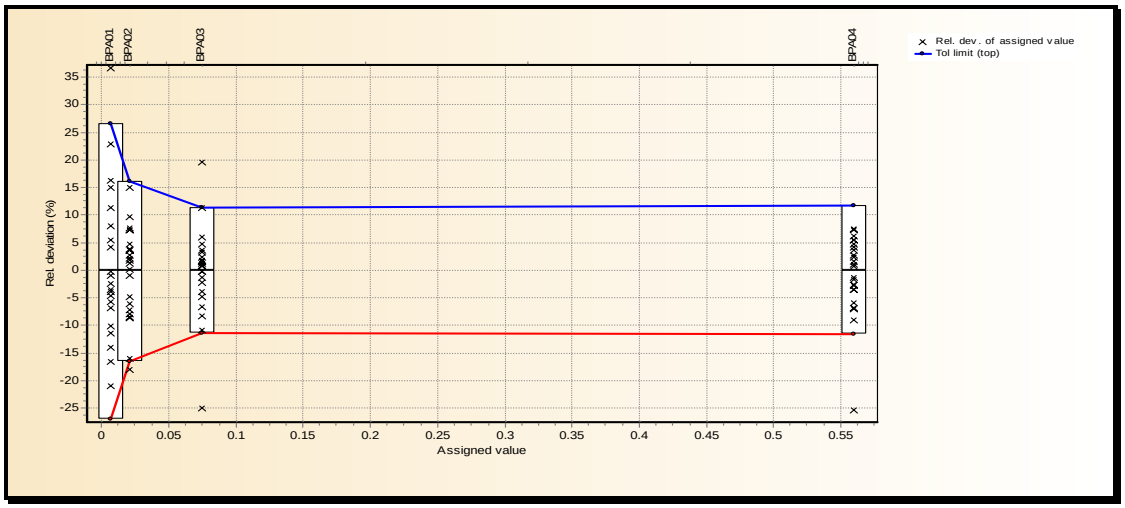
Figure 8 represent the overall z-score distribution for all the 91 measurand-matrix-laboratory combination for DIDP in 4 samples and 24 laboratories'. Figure 9 represent them in histogram, like Kernel density plot and normal distribution plot - showing its real normal distribution.

Figure 5. Tolerance limits in relation with the concentration

a) z-scores calculated based on target SD = 0.5*Horwitz;



a) z-scores calculated based on target SD = reproducibility SD of the ILC02 (calculated according to the robust 5275-5 algorithm.



As a conclusion of the precision exercise on the quantification of Bisphenol A in the new milk simulant 50% ethanol, this ILC showed that:

- The participation in the ILC was satisfactory for the purpose of the study towards validation with 24 laboratories.
- The validation of the method based on HPLC-FLD according to the description based mostly on the previous CEN standard TS 13130-13 was successful with more than 8 valid results thanks to the proactive involvement of the NRLs.
- The precision that could be suggested are those listed as parameters in table 5, reported again below

Concentration level mg/kg	Reproducibility (R), %	Horrat R	Repeatability (r), %
0.0067	15	0.7	6
0.021	10	0.4	4
0.075	6	0.3	2
0.56	6	0.3	0.8

- With respect to the scarcity of data previously available in the validation performed as reported in the CEN standard TS 13130-13 (issued version of 2005), this validation also provides a great breadth of valuable detailed and traceable raw data, which should prove extremely relevant for the creation of a potential standard from CEN.

****

Figure 6 a - Youden plot BPA01 against BPA02 –in low level calibration range

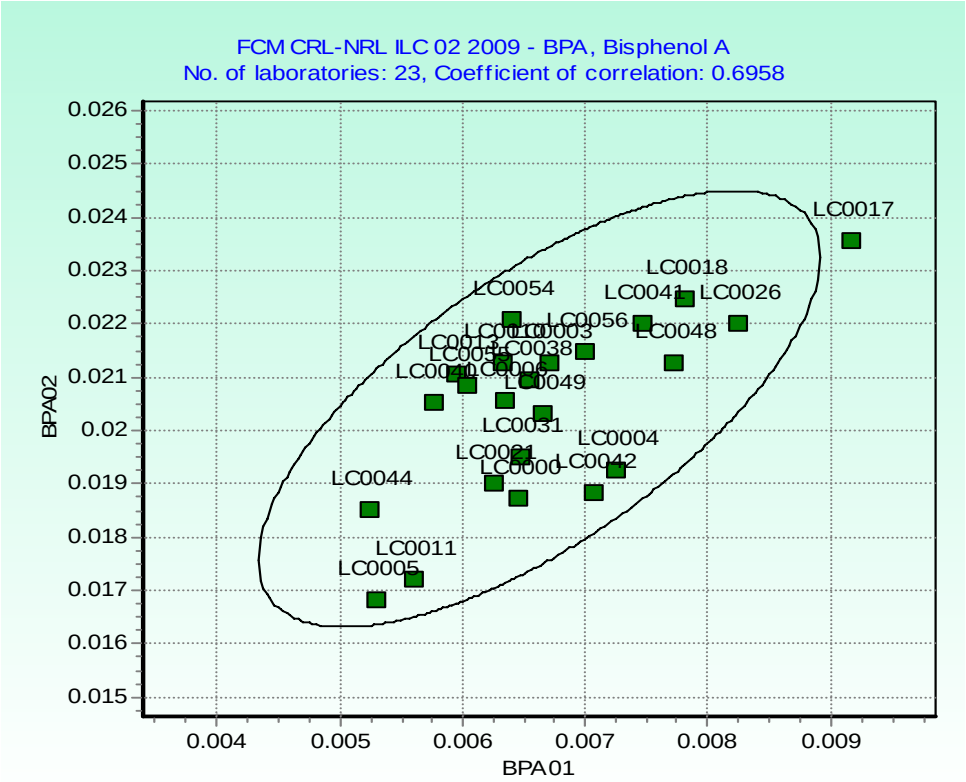


Figure 6 b - Yoden plot BPA03 against BPA04 –in high level calibration range

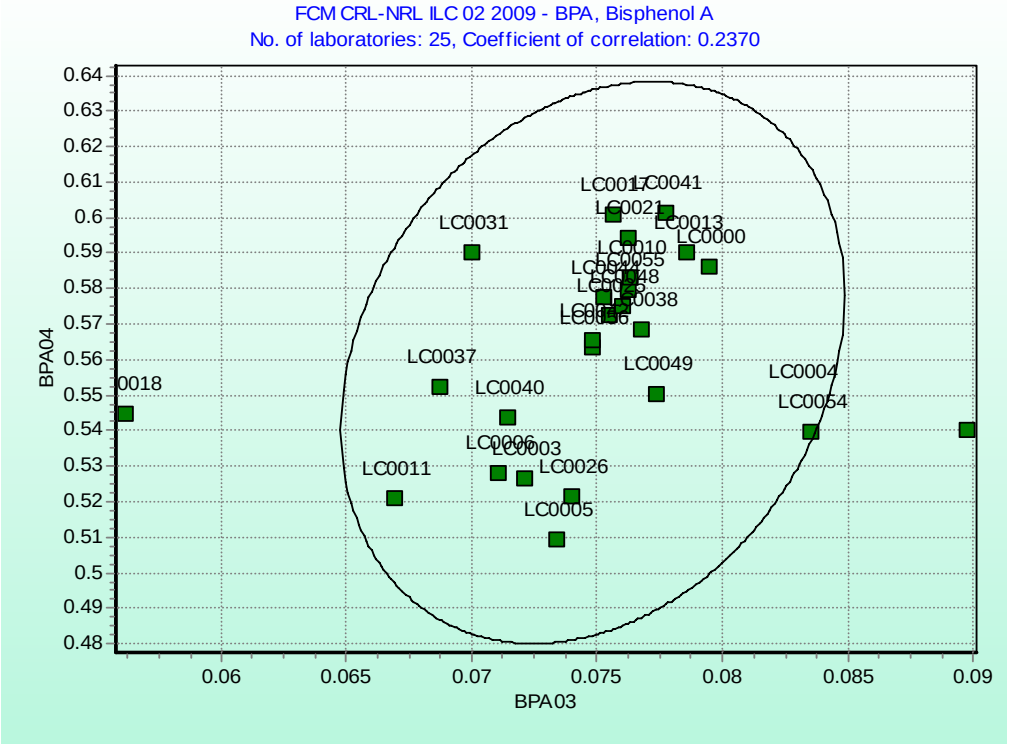


Figure 7a Plot of laboratories mean values against their repeatability SD for BPA01

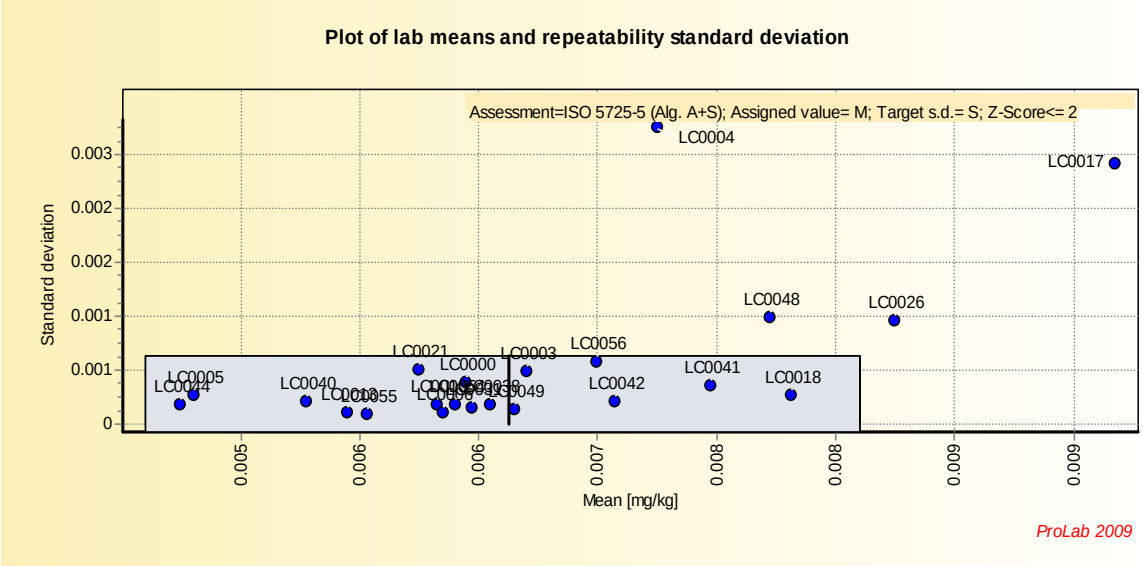


Figure 7b Plot of laboratories mean values against their repeatability SD for BPA02

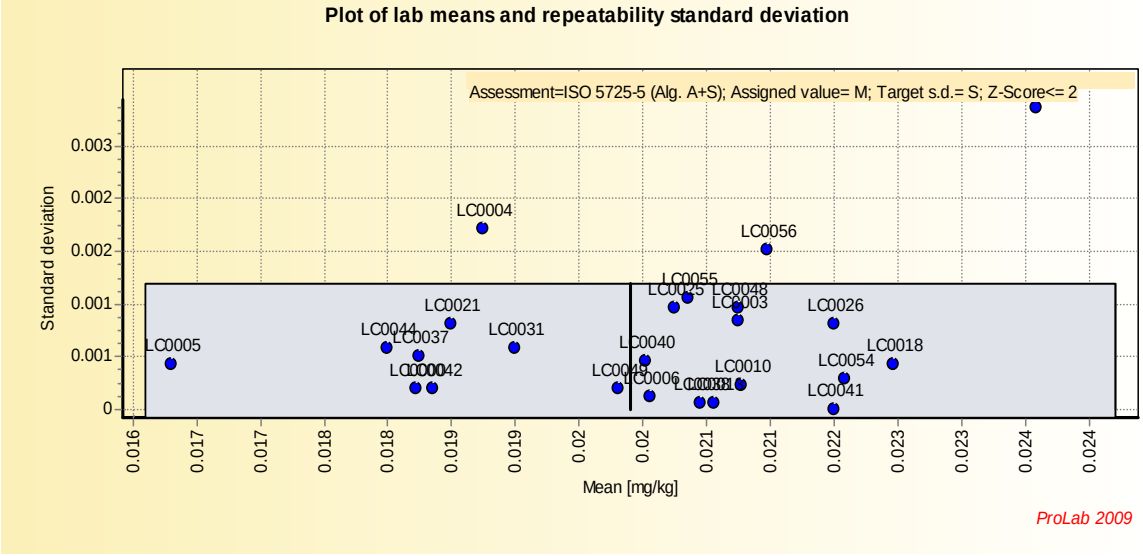


Figure 7c Plot of laboratories mean values against their repeatability SD for BPA03

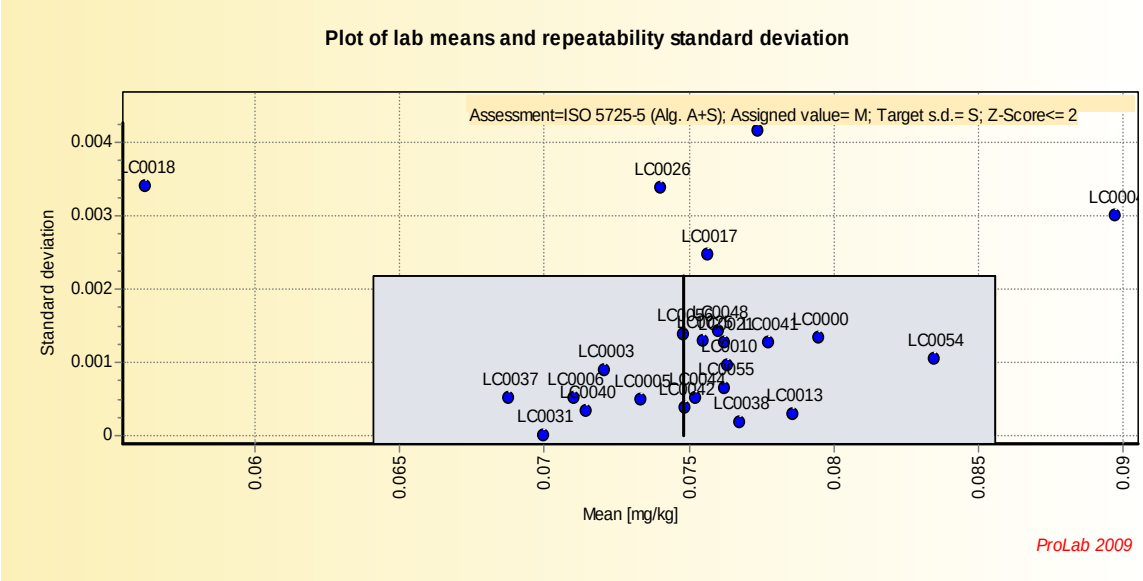


Figure 7d Plot of laboratories mean values against their repeatability SD for BPA04

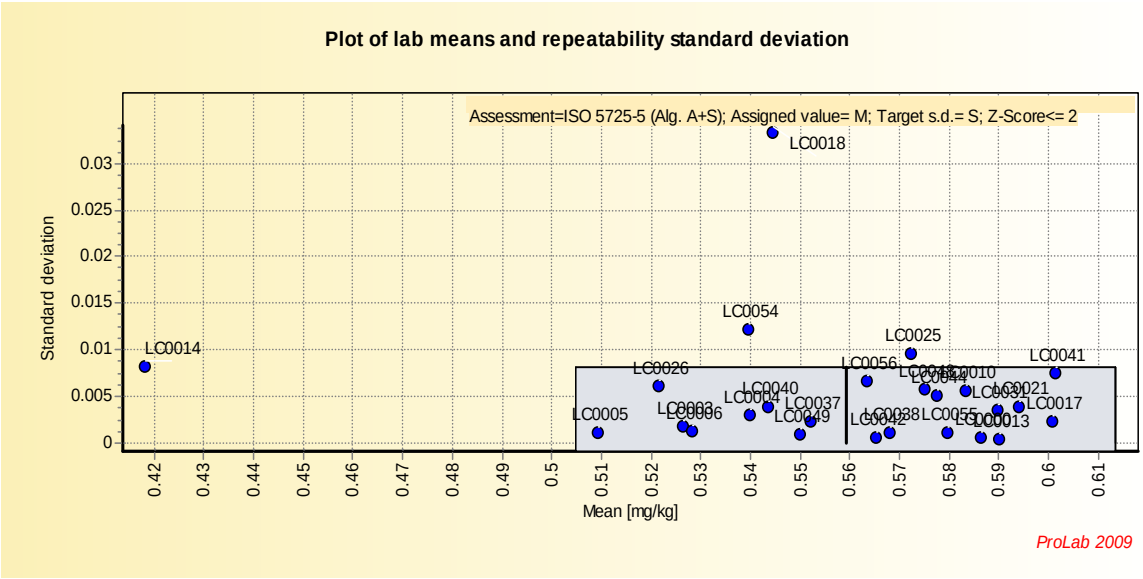
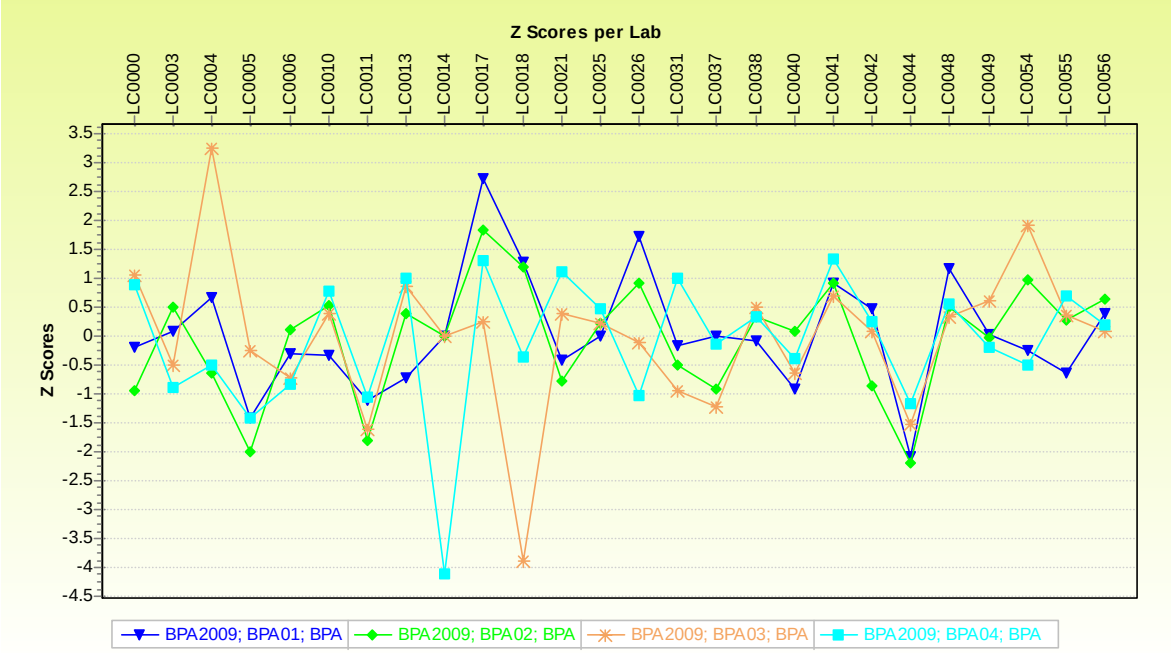


Figure 8:. Distribution of all z-score – scatter

a) distribution by laboratories



b) distribution by samples

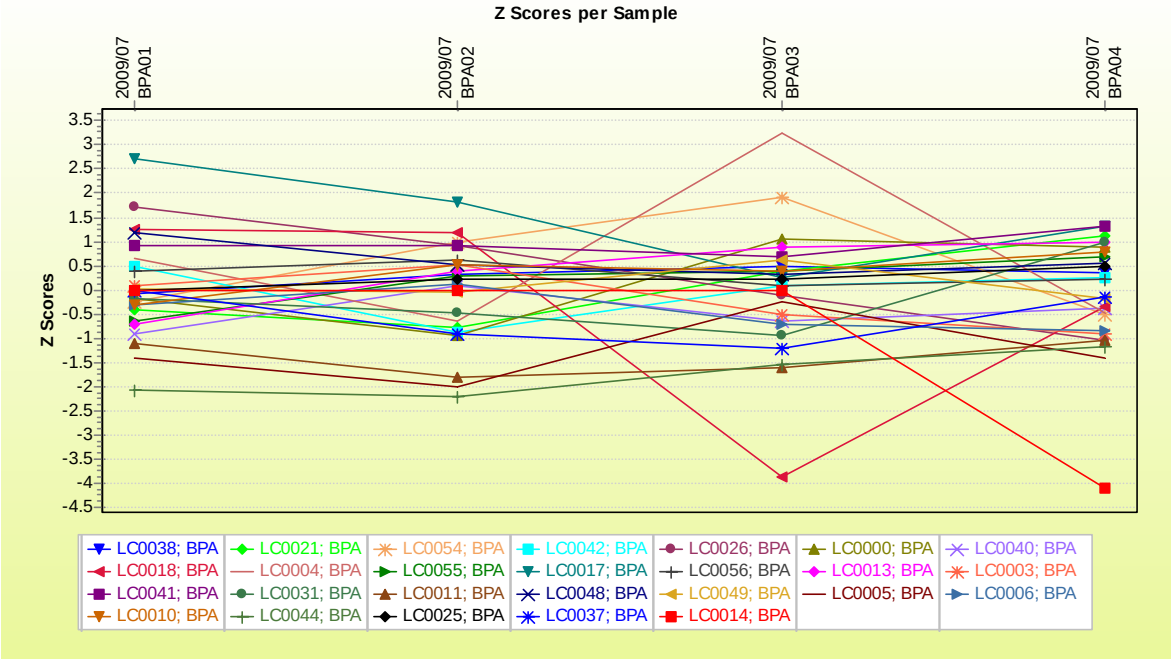
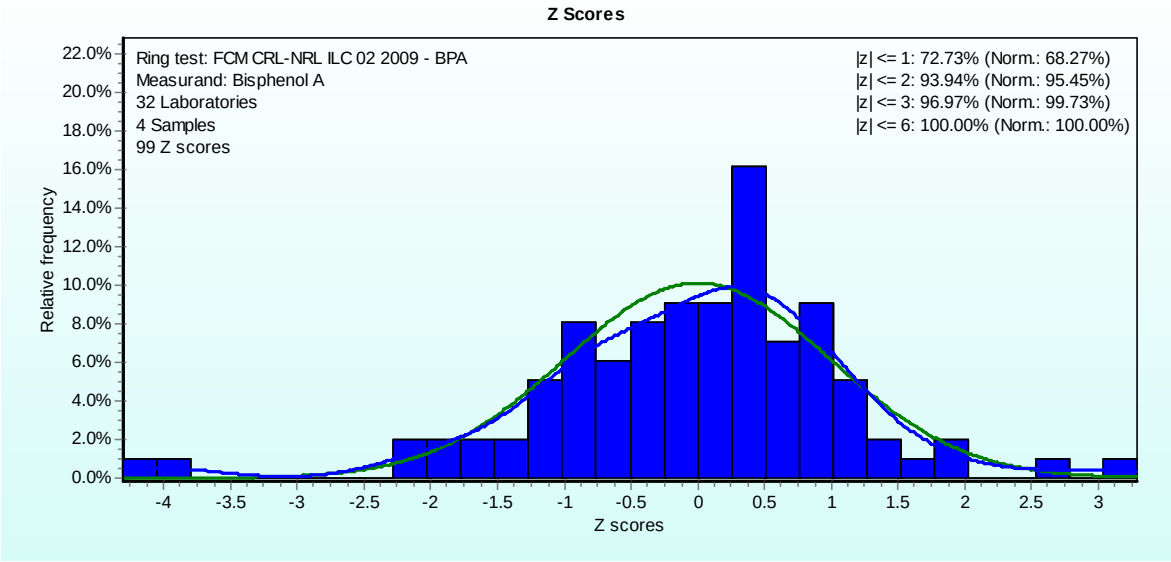


Figure 9:. Distribution of all z score histogram (blue bars), Kernel density plot (blue line) and normal distribution plot (green line)



9. Acknowledgements

The NRLs and guests participating in this exercise - listed below - are kindly acknowledged.

AUSTRIA	Austrian Agency for Health and Food Safety (AGES)
BELGIUM	Institute of Public Health, ISSP-LP
REPUBLIC OF CYPRUS	Laboratory for Control of Food Contact Materials and Control of Toys Ministry of Health, State General Laboratory (SGL)
CZECH REPUBLIC	NIPH- NRL for Food Contact Materials and for Articles for children under 3 years old, National Institute of Public Health (SZU')
ESTONIA	Health Protection Inspectorate - Central Laboratory of Chemistry
FINLAND	Finnish Customs Laboratory
FRANCE	Center for Energy Material and Packaging - Laboratoire National d'Essais
FRANCE	SCL Laboratoire de Bordeaux-Pessac
GERMANY	Bundesinstitut für Risikobewertung (BFR) (Federal Institute for Risk Assessment)
GREECE	General Chemical State Laboratory, D' Chemical Service of Athens, Section Laboratory of Articles and Materials in Contact with Foodstuffs
IRELAND	Public Analyst Laboratory - Sir Patrick Duns Hospital
LUXEMBOURG	Laboratoire National de Sante', Division du Controle des denrées alimentaires
POLAND	Laboratory of Department of Food and Consumer Articles Research , National Institute of Hygiene
PORTUGAL	ESB-SE (Portuguese Catholic University - Biotechnology College – Packaging Department)
SLOVAK Republic	National Reference Centre and Laboratory for material and articles intended to come into contact with food, Regional Public Health Authority In Slovak Republik
SLOVENIA	National Institute of Public Health of Republic of Slovenia, Dept of Sanitary Chemistry
SPAIN	Centro Nacional de Alimentación, Agencia Espanola de Seguridad Alimentaria y Nutrición (AESAN)
THE NETHERLANDS	Food and Consumer Product Safety Authority (VWA), Inspectorate for Health Protection region North
UK	Central Science Laboratory
Germany	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Bavarian Health and Food Safety Authority
Germany	Central Institute of the German Armed Forces Medical Service Koblenz - Department III Food ChemistryHessisches Landeslabor LHL Standort Wiesbaden
Germany	Landeslabor Berlin-Brandenburg
Spain	CNTA - Laboratorio del Ebro
Spain	Laboratorio de Salud Pública de Lugo - Consellería de Sanidad - Xunta de Galicia
Spain	Laboratori Agencia Salut Publica de Barcelona

10 References

[1] ISO 13528:2005; Statistical Methods for Use in Proficiency Testing by Interlaboratory Comparisons

[2] M. Thompson, *Analyst*, (2000), 125, 385-386.

[3] (EC) No 372/2007 Commission Regulation of 2 April 2007 laying down transitional migration limits for plasticisers in gaskets in lids intended to come into contact with foods.

[4] 2002/72/EC Commission Directive of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs. (Plastics: Unofficial consolidated version including 2002/72/EC, 2004/1/EC, 2004/19/EC, 2005/79/EC, 2007/19/EC, 2008/39/EC)

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[7] DIN 38402 A45 Ringversuche zur externen Qualitätskontrolle von Laboratorien.

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[9] T. Linsinger *et al.*, *Accreditation and Quality Assurance in Analytical Chemistry* (2001), 6, 20-25

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[11] ISO 5725-2:1994 (E) Accuracy of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method

[12] AMC, *Representing data distributions with kernel density estimates*. AMC Technical Brief, 2006, http://www.rsc.org/images/brief4_tcm18-25925.pdf.

[13] Lowthian, P.J. and M. Thompson, *Bump-Hunting for the proficiency tester - searching for multimodality.* The Analyst, 2002. 127: p. 1359, https://www.swetswise.com/eAccess/viewAbstract.do?articleID=14625081.

[14] ISO 5725 -2 Accuracy (trueness and precision) of measurement method and results. Part 2: Basic method for the determination of repeatability and

reproducibility of a standard measurement method.

[15] ISO 5725 -5 Accuracy (trueness and precision) of measurement method and results. Part 5: Alternative methods for the determination of the precision of a standard measurement method.

[16] ISO GUIDE 35:2006; Reference materials – General and statistical principles for certification.

11. Annexes

Annex 1 Standard Operating Procedure: Determination of Bisphenol A in 50% aqueous ethanol.

Annex 2: Invitation letter to laboratories ILC 2009/02

Annex 3: Format for confirmation of participation to ILC 2009/02

Annex 4: Letter accompanying the samples ILC 2009/02.

Annex 5: Letters of confirmation of receipt ILC 2009/02

Annex 6: Instruction for the compilation of the results in electronic format.

Annex 7: Format for the compilation of results in non-electronic format.

Annex 8 Summary of laboratories participation in interlaboratory comparison exercises

Annex.9 Procedure for the preparation of the spike of Bisphenol A in 50% aqueous ethanol for ILC 2009/02

Annex 10: Results of the homogeneity study.

Annex 11: Results of the stability study.

Annex 1

Standard Operating Procedure:

Determination of Bisphenol A in 50% aqueous ethanol.

Materials and articles in contact with foodstuffs — Plastics substances subject to limitation — Determination of 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A) in 50% ethanol:water food simulant

Introduction

2,2-Bis(4-hydroxyphenyl)propane, herein referred to as Bisphenol A, $C_{15}H_{16}O_2$, PM/Ref. 13480, is a monomer used in the manufacture of certain plastics materials and articles intended to come into contact with foodstuffs. After manufacture residual Bisphenol A can remain in the finished product and may migrate into foodstuffs coming into contact with that product.

1 Scope

This document, modified EN 13130 part 13, specifies a method for the determination of Bisphenol A in a volume fraction of 50 % ethanol aqueous food simulant. The level of Bisphenol A monomer determined is expressed as milligrams Bisphenol A per kilogram of food simulant. The method is applicable to the quantitative determination of Bisphenol A at a minimum level of 0,002 mg/kg in 50% ethanol food simulant.

2 Principle

The level of Bisphenol A in 50 % ethanol aqueous food simulant is determined by high performance liquid chromatography (HPLC) with fluorescence (FLD) detection. Calibration is achieved by analysis of relevant simulant containing known amounts of Bisphenol A.

3 Reagents

NOTE All reagents should be of recognised analytical quality unless otherwise stated

3.1 Analyte

2,2-bis(4-hydroxyphenyl)propane (Bisphenol A or 4,4'-(methylethylidene)-bisphenol or 4,4'-isopropylidenediphenol), $C_{15}H_{16}O_2$, molecular weight: 228,28, purity >99 %

3.2 Chemicals

3.2.1 Methanol, HPLC grade

3.2.2 Water, deionised

3.2.3 Ethanol, HPLC grade

3.3 Solutions

3.3.1 Mobile phase for HPLC, methanol/water = 70:30

Measure 500 ml of methanol (3.2.1) and 215 ml of water (3.2.2) and mix.

3.3.2 Stock solution of Bisphenol A in methanol at a defined concentration of approximately 0,75 mg/ml

Weigh to the nearest 0,1 mg approximately 75 mg of Bisphenol A (3.1) into a 100 ml volumetric flask. Dissolve the Bisphenol A in methanol (3.2.1) and make up to the mark with methanol at 20°C as the flask are calibrated at 20°C.

Calculate the concentration in milligram of Bisphenol A per millilitre of solution.

Store the solution in a well closed container in the dark for a maximum period of 3 months at any temperature between +20 °C and –20 °C.

Repeat the procedure to provide a second stock solution. The two solutions should be cross-checked against one another. However, if other in-house quality systems are in place then these may be applicable instead.

3.3.3 Standard solution of Bisphenol A in methanol at a defined concentration of approximately 0,075 mg/ml

Transfer 2 ml of the stock solution of Bisphenol A (3.3.2) into a 20 ml volumetric flask and make up to the mark with methanol at 20°C (3.2.1).

Calculate the concentration in milligram Bisphenol A per millilitre of solution
.
Repeat the procedure to obtain a second standard solution if applicable.

3.3.4 Dilute solution of Bisphenol A in methanol at a defined concentration of approximately 0,0075 mg/ml

Transfer 2 ml of the standard solution of Bisphenol A (3.3.3) into a 20 ml volumetric flask and make up to the mark with methanol at 20°C(3.2.1).

Calculate the concentration in milligram Bisphenol A per millilitre of solution.

Repeat the procedure to obtain a second dilute solution if applicable.

4 Apparatus

NOTE An instrument or item of apparatus is listed only where it is special or made to a particular specification, usual laboratory glassware and equipment being assumed to be available.

4.1 High performance liquid chromatograph, preferably, equipped with an automatic 20 µl loop injector and a fluorescence detector connected to an integrator.

4.2 HPLC column, capable of separating Bisphenol A fully from peaks originating from the simulant and/or solvents used.

Appropriate operating conditions shall be established for the specific equipment used for the determination.

NOTE For guidance, column and the parameters established for the column selected are as follows:.

column:	stainless steel 150 × 3.0 mm packed with C18 coated spherical silica gel, particle size 5 µm, (load of 17,5 % carbon and end-capped)
mobile phase:	methanol/water 70 : 30 (3.3.1)
injection volume:	20 ul
flow rate:	0.5 ml/min
detection:	fluorescence detection
excitation:	235 nm
emission:	317 nm

Alternative fluorescence excitation and emission wavelengths are:

Excitation:	275 nm
Emission:	305 nm

4.3 Mechanical shaker (Vortex)

4.4 Micro syringes, volume 10 µl, 50 µl, 100 µl, 250 µl and 500 µl

4.5 Volumetric flasks, volume 20 ml, 25 ml, 100 ml

5 Samples

5.1 Test sample preparation

Samples shall be kept refrigerated at +4 °C in closed containers with the exclusion of light. Analyte-free samples of relevant food simulant of the same type as those to be analysed shall also be prepared for calibration purposes.

Transfer approximately 1 ml of the food simulant obtained from the migration experiment (see EN 13130-1) into a vial suitable for HPLC injections.

5.2 Blank sample preparation

Treat food simulant which have not been in contact with packaging material in the same way as described in 5.1.

5.3 Calibration sample preparation

5.3.1 The calibration range, applicable to the determination of Bisphenol A at concentrations between 0,030 mg and 1,2 mg of Bisphenol A per litre of food simulant according to pr EN 13130 : 2006 for compliance assessment

Transfer with a micro syringe (4.4) into a series of eight 25 ml volumetric flasks (4.5) 0 µl, 10 µl, 20 µl, 40 µl, 100 µl, 200 µl, 300 µl and 400 µl of the standard Bisphenol A solution (3.3.3), make up to the mark with volume fraction of 50 % aqueous ethanol, and mix thoroughly. The calibration solutions thus obtained contain 0 □g/ml and approximately 0,030 mg, 0,060 mg, 0,12 mg, 0,30 mg, 0,60 mg, 0,90 mg or 1,2 mg of Bisphenol A per litre of food simulant.

Calculate the exact concentrations of Bisphenol A in the calibration samples in milligram per litre

5.3.2 The calibration range, applicable to the determination of Bisphenol A at concentrations between 0.002 mg and 0.030 mg of Bisphenol A per litre of food simulant for exposure assessment purposes

Transfer with a micro syringe (4.4) into a series of eight 25 ml volumetric flasks (4.5) 0 µl, 6 ul*, 10 µl, 20 µl, 30 µl, 40 µl, 60 µl, 80 µl and 100 µl of the standard Bisphenol A solution (3.3.4), make up to the mark with volume fraction of 50 % aqueous ethanol, and mix thoroughly. The calibration solutions thus obtained contain 0 µg/ml and approximately 0.0018 mg, 0.003 mg, 0.006 mg, 0.009 mg, 0.012 mg, 0.018 mg, 0.024 mg or 0.030 mg of Bisphenol A per litre of food simulant.

Calculate the exact concentrations of Bisphenol A in the calibration samples in milligram per litre.

* optional

6 Procedure

6.1 HPLC analysis

When starting measurements, examine the baseline stability and response linearity of the detector.

The fluorescence detector shall be able to detect 0,04 ng on column of Bisphenol A (20 ul from 0.002 mg/l) at a signal to noise ratio of 3:1.

The same operating conditions of the HPLC system shall be maintained throughout the measurements of all sample and calibration solutions. Each test solution shall be injected, at least, in duplicate.

6.2 Calibration

Analyze each of the calibration solutions as prepared in 5.3.1 and 5.3.2. Measure the peak height or area of Bisphenol A. Construct calibration curves plotting these values against the concentration of Bisphenol A in milligrams per litre in the calibration solutions.

The calibration curves shall be rectilinear and the correlation coefficient should be 0,998 or better.

6.3 Execution of determination

Analyze the test sample solutions, prepared as described in 5.1 and 5.2, applying the HPLC conditions used for the calibration solutions. Measure the peak height or area of Bisphenol A and either read the Bisphenol A concentration, in milligrams per litre, from the calibration curve or calculate it from the regression coefficient.

Re-calculate the concentration of Bisphenol A in 50% ethanol in mg/kg without making the conventional assumption that the density of food simulant is 1, but taking into consideration the real density of the food simulant at the temperature of analysis of the samples !

6.4 HPLC interferences

Following the method described, no interferences were detected in 50% aqueous ethanol food simulant.

7 Expression of results

7.1 Calculation of analyte level

NOTE The following calculations assume that for all measurements exactly the same mass or volume of food simulant has been used.

7.1.1 Graphical determination

Calculate the average of peak area/height values obtained from the test samples in accordance with 6.3 and read the Bisphenol A concentration of the test samples from the calibration graph (6.2)

7.1.2 Calculation from the regression parameters:

If the regression line equation is:

$$y = (a \times x) + b$$

where

y is the peak area of Bisphenol A;

a is the slope of the regression line ;

x is the concentration of Bisphenol A in the food simulant in milligrams per litre;

b is the intercept of the regression line,

the concentration of Bisphenol A in the food simulant is given by:

$$C_{BISfs} = \frac{y - b}{a}$$

where

C_{BISfs} *is the concentration of Bisphenol A in milligrams per litre of food simulant*

Both procedures yield directly the Bisphenol A concentration in the food simulant in milligrams per litre

The method applying calculation from the regression parameters is the preferred method

The concentration should be recalculated from mg/l to mg/kg considering the real density of the 50% ethanol aqueous simulant at the corresponding temperature.

Annex 2: Invitation letter to laboratories ILC 2009/02.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
CRL
Food Contact Materials

Ispra July 13, 2009
I02-CAT/CS/sm(2009)
Ares(2009)171288

Dear Madam, Sir

Comparative trial 2009 ILC 2009 - 02 from CRL FOOD CONTACT MATERIALS "Determination of Bisphenol A in 50% aqueous ethanol as food simulant"

On behalf of the CRL for food contact materials, I would like to invite you to participate in a comparative trial/interlaboratory comparison (ILC) exercise for the determination of Bisphenol A in 50% aqueous ethanol which is due to start in the end of august. Please note that according to the agreement of the December's CRL-NRL FCM plenary, this year the ILC exercise is a validation study of the quantification method in the milk simulant. Each participant will be asked to follow strictly the method description (SOP) which is equal to the working document prEN 13130-13:2006 prepared by CEN/TC194/SC1/WG2 and which will supersede CEN/TS 13130-13:2005. Both methods will be sent to you in electronic format and the SOP will be provided in the kit as well.

I would like to remind you that it is a duty for you as an NRL-FCM to participate in the ILCs organised by the CRL-FCM since the work programme is decided with your agreement. For this reason we encourage all of you to actively participate in this exercise. There is no charge for participation. Feel free to involve your local controls.

We have pre-registered everyone, which means we will send test kits to all of you. We however need to receive the **proformat of your participation** for our own administrative purposes. Kindly send back the proformat **by September 05** to: Catherine Simoneau (catherine.simoneau@jrc.ec.europa.eu)**.** If you need more test kits to involve more laboratories at the national level we have another 20 kits of test materials for Bisphenol A in 50% aqueous ethanol. In this case please let me know immediately by e-mail so we can pack accordingly.

The samples will be sent to you by the **end of august**. You will find additional information in the kit sent and on the form "shipment test BPA". You will also receive more detailed instructions for the compilation of the results. The deadline for submission of results is **12th October 2008.**

If you have any question, please contact Catherine Simoneau (catherine.simoneau@jrc.ec.europa.eu), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau

Dr. Catherine Simoneau
Operating Manager, Community Reference Laboratory for Food Contact Materials
European Commission, DG-Joint Research Centre
Institute for Health and Consumer Protection
Unit Physical and Chemical Exposure, T.P. 260
Ispra Va 21020 Italy

Cc: MM. D. Kotzias (JRC), D. Sarigiannis (JRC), B. Larsen (JRC), F. d'Atri (SANCO)
Mrs. A Schaefer (SANCO)

Annex 3: Format for confirmation of partecipation to ILC 2009/02.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
CRL
Food Contact Materials

Ispra July 13, 2009
Annex to I02-CAT/CS/sm(2009)

Participation to CRL-FCM ILC 2009 - 02
Interlaboratory comparison (ILC) exercise
for the determination of Bisphenol A in 50% aqueous ethanol

CONFIRMATION OF PARTICIPATION

Your Name:	
Organization:	
Address:	
E-mail:	
Phone:	

item	YES	NO
I will participate the collaborative trial on analysis of Bisphenol A in 50% aqueous ethanol and will deliver results on time		
I have already the package with the files for filling the results and especially RingDat3.exe file from last year and I need only lab files for this year's ILC		
I don't have the package with the files for filling the results from last year		

Kindly send back this proformat to: Catherine Simoneau (catherine.simoneau@jrc.it) **by September 5th .**
The samples will be sent to you by the end of august. You will find additional information in the kit sent. The deadline for submission of results is **12th October 2009**

If you have any question, please contact Catherine Simoneau (catherine.simoneau@jrc.it), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau

Annex 4: Letter accompanying the sample ILC 2009/02.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra August 18, 2009
Appendix to I02-CAT/CS/sm(2009)
Ares(2009)171288

Shipping kit for interlaboratory comparative testing CRL-FCM ILC02 2009 – Bisphenol A in 50% aqueous ethanol

Shipping kit - Bisphenol A in 50% aqueous ethanol:

- four 40 ml screw cap vials containing about 30 ml of 50% aqueous ethanol spiked with Bisphenol A at 4 concentration levels.

Documentation:

- instruction for the compilation of results;
- letters of confirmation of receipt ILC 02 2009;
- empty form "Test results" for filling the results;
- method of analysis - SOP for Bisphenol A in 50% aqueous ethanol;

Results requested:

o result format: Use the personal "NAME.LAB" file that will be provided by e-mail;
o use the printed empty form "Test results" in case of difficulties with the files;

Annex 5: Letter of confirmation of receipt of ILC 2009/02.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra August 18, 2009
Appendix to I02-CAT/CS/sm (2009)

PARTICIPATION TO CRL-FCM ILC02 2009
BISPHENOL A IN 50% AQUEOUS ETHANOL

CONFIRMATION OF RECEIPT OF THE SAMPLES

Please return this form to confirm that the sample package has arrived. In case the package is damaged, please state this on the form and contact us immediately.

Your Name:	
Organization:	
Address:	
E-mail:	
Phone:	

Any remarks ……

Date arrival package ……

Signature ……

Kindly send back this form to: Catherine Simoneau (catherine.simoneau@jrc.it).

Sincerely yours,

Catherine Simoneau

Dr. Catherine Simoneau
Operating Manager, Community Reference Laboratory for Food Contact Materials
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Annex 6: Instructions for the compilation of the results in electronic format.

EUROPEAN COMMISSION
GENERAL DIRECTORATE JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection – IHCP
Unit Chemical Assessment and Testing

Community Reference Laboratory
Food Contact Materials

Ispra august 18, 2009
Appendix to I02-CAT/CS/sm(2009)

Instructions for the compilation of the results for interlaboratory comparative testing CRL-FCM ILC01 2009

DEADLINE: Monday, October 12th

Results requested:

Perform four replicates for each sample and report all the four replicate data using the unit of measure specified in the lab file or printed paper "Test results":

Compilation of results

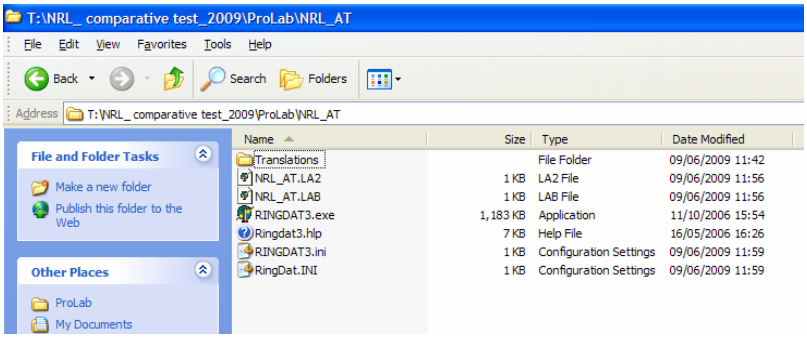
Data generated by the laboratories for the comparative test CRL-FCM PT001 will be processed by the CRL-FCM using a software package for statistical analyses and professional data handling of interlaboratory tests.

- a simple data entry program (RingDat3.exe) is provided to each participating laboratory (from last year PT 2008 or now on CD-ROM 2009) ;
- two additional lab files with the extension "participant.Lab" and "participant.LA2", generated by the ProLab software are provided to each laboratory individually (personal files) by e-mail;
- the name of each laboratory and the samples are codified by the software, so that each participant will receive a sample with unique codified numbers (i.e., 0586);
- The "*.LA2" file contains information about the participant – laboratory name and laboratory code;
- The "*.LAB" file is unique to each laboratory (personal) and contains information about the samples (samples code) and measurands that have to be analysed and reported.

Each laboratory has to start the RingDat3.exe program and to open "name.LAB" file for reporting the results. A table will appear with cells for every measurand/sample combination. That is why 6 sample codes will appear for each participant/file - 3 for acetonitrile and 3 for oil samples.

Procedure to be followed by laboratories for the opening the LAB files from the software.

First: Create a folder on your computer and transfer RingDat files and Translation directory (from last year PT2008 or from CD-ROM 2009) into it. Copy there as well the "NRL_X.LAB" and "NRL_X".LA2 files which you'll receive by mail. The folder should contain:



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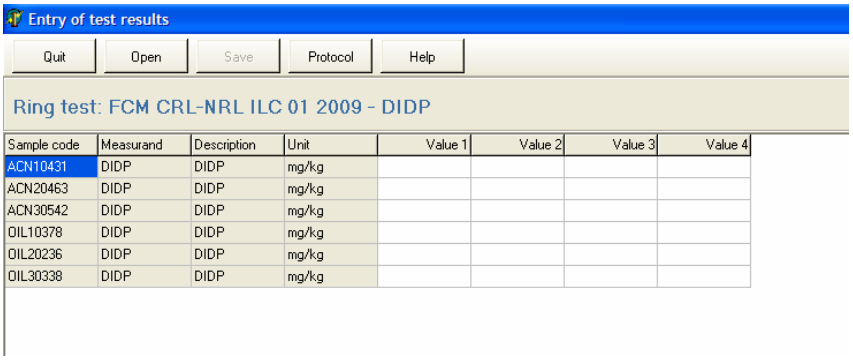
Then:

- Open the file "RINGDAT3.exe"
- Click on "Open" command
- Select the "NRL_X.LAB" file (where X is the member state abbreviation- with one to three letters) and click on "Open" command using the button on the top menu of the window

- Windows you should see using the software is :



Sample code	Measurand	Description	Unit	Value 1	Value 2	Value 3	Value 4
ACN10431	DIDP	DIDP	mg/kg				
ACN20463	DIDP	DIDP	mg/kg				
ACN30542	DIDP	DIDP	mg/kg				
OIL10378	DIDP	DIDP	mg/kg				
OIL20236	DIDP	DIDP	mg/kg				
OIL30338	DIDP	DIDP	mg/kg				

- Fill the table with your data
- Save the file using the button on the top menu of the window
- Send only the "NRL_X.LAB" file by e-mail to catherine.simoneau@jrc.it

Please fill your results and send it back by e-mail to Catherine Simoneau (catherine.simoneau@jrc.it) by 12th October 2009.

If you have any question, please contact Catherine Simoneau (catherine.simoneau@jrc.it), ph. +39.0332.785889

Sincerely yours,

Catherine Simoneau
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Annex 7: Form for the compilation of the results in non-electronic format.

Ring test: FCM CRL-NRL ILC 02 2009 - BPA

Test results

Lab.-code: LC0042

Sample code	Measurand	Description	Unit	Value 1	Value 2	Value 3	Value 4
10226	BPA	Bisphenol A	mg/kg				
20056	BPA	Bisphenol A	mg/kg				
30054	BPA	Bisphenol A	mg/kg				
40110	BPA	Bisphenol A	mg/kg				

....

Place and date | Manager of laboratory (in block letters) | Signature

Annex 8 Summary of laboratories participating in interlaboratory comparison exercise

Member State	Name of NRL/PARTICIPANT
AUSTRIA	Austrian Agency for Health and Food Safety (AGES),
BELGIUM	Institute of Public Health, ISSP-LP
REPUBLIC OF CYPRUS	Laboratory for Control of Food Contact Materials and Control of Toys Ministry of Health, State General Laboratory (SGL)
CZECH REPUBLIC	NIPH- NRL for Food Contact Materials and for Articles for children under 3 years old, National Institute of Public Health (SZU')
ESTONIA	Health Protection Inspectorate - Central Laboratory of Chemistry
FINLAND	Finnish Customs Laboratory
FRANCE	Center for Energy Material and Packaging - Laboratoire National d'Essais
FRANCE	SCL Laboratoire de Bordeaux-Pessac
GERMANY	Bundesinstitut für Risikobewertung (BFR) (Federal Institute for Risk Assessment)
GREECE	General Chemical State Laboratory, D' Chemical Service of Athens, Section, Laboratory of Articles and Materials in Contact with Foodstuffs
IRELAND	Public Analyst Laboratory - Sir Patrick Duns Hospital
LUXEMBOURG	Laboratoire National de Sante', Division du Controle des denrées alimentaires
POLAND	Laboratory of Department of Food and Consumer Articles Research , National Institute of Hygiene,
PORTUGAL	ESB-SE (Portuguese Catholic University - Biotechnology College – Packaging Department)
SLOVAK REPUBLIC	National Reference Centre and Laboratory for material and articles intended to come into contact with food, Regional Public Health Authority In
SLOVENIA	National Institute of Public Health of Republic of Slovenia, Dept of Sanitary Chemistry
SPAIN	Centro Nacional de Alimentación, Agencia Espanola de Seguridad Alimentaria y Nutrición (AESAN)
THE NETHERLANDS	Food and Consumer Product Safety Authority (VWA), Inspectorate for Health Protection region North
UK	Central Science Laboratory
Germany	Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit Bavarian Health and Food Safety Authority
Germany	Central Institute of the German Armed Forces Medical Service Koblenz - Department III Food ChemistryHessisches Landeslabor LHL Standort Wiesbaden
Germany	Landeslabor Berlin-Brandenburg
Spain	CNTA - Laboratorio del Ebro
Spain	Laboratorio de Salud Pública de Lugo - Consellería de Sanidad - Xunta de Galicia
Spain	Laboratori Agencia Salut Publica de Barcelona

Annex 9

Procedure for the preparation of the spike of BPA in 50% aqueous ethanol for ILC.

1. Washing of the mixing bottles:

- 5 L mixing bottles were washed in a washing machine, then filled with 1 L of deionised water (resistivity 18.0 MΩ.cm @ 25 °C) and mixed.
- The water was removed.
- The tank was filled with 1 L of ethanol (Fluka HPLC grade) and mixed for 2 hours.
- Ethanol was removed. The internal surface of the tank and the mixing device were rinsed with ethanol.
- The tank was filled with 1 L of n-hexane (Sigma-Aldrich HPLC grade) and mixed for 2 hours.
- n-hexane was removed. The internal surface of the tank and the mixing device were rinsed with n-hexane.
- The tanks and the mixing device were dried with a stream of pure nitrogen.

2. Washing of the glassware:

The glassware (40 ml clear glass screw cap vials) was washed in a washing machine, then rinsed with deionised water (resistivity 18.0 MΩ.cm @ 25 °C). Test was performed on a pre-washed vial with a portion of 50% aqueous ethanol. Blank was confirmed as free of BPA interference.

Preparation of stock solution of BPA in methanol (approx. 750 mg/l)

- The balance (balance AX 205) was checked against calibrated weight in the range 1000 mg + flask
- 77.50 mg of BPA Aldrich was weighted into a 100 ml class A volumetric flask and dissolved in termostated to 20ºC methanol, the flask was filled up to the mark.
- concentration of the resulting stock solution is 0.775 mg/ml.

Preparation of intermediate solution of BPA in methanol (approx. 75 mg/l)

- 2 ml of the BPA stock solution were transferred into a 20 ml class A volumetric flask, by mean of a class A pipette. The flask was filled to the mark with termostated to 20ºC methanol.
- concentration of the resulting intermediate solution is 0.0775 mg/ml.

3. Preparation of BPA in 50% aqueous ethanol

- The balance (technical up to 6 kg) was checked by weighting 1000 ml deionised water at 20ºC, obtaining a value of 996.4 g against 997.5 as it should be according to the t coefficient of expansion of water, and with 100 and 200 g calibrated weights.

- The empty 5 l mixing bottle was weighted (P1) with the screw cap.
- An aliquot of 1 l of simulant 50%acqueous ethanol was transferred into the 5 l mixing bottle.
- The calculated spiking volume (table 1) from stock solution of 0.775 mg/ml or intermediate solution of 0.0775 mg/ml was transferred into the mixing bottle containing the simulant.
- The mixing bottle was filled with simulant up to a total weight of 2000 g. The bottle containing the spiked simulant was weighted (P2) and the weight of simulant calculated: P3= P2-P1.
- The bottle was mixed
- The spiked simulant was distributed in screw cap 40 mL vials directly from the mixing bottle, via the stopcock positioned on the bottom of the bottle (40 ml aliquots).

Note: all the weights were repeated 3 times and the average value was calculated.

Table 8 Reference concentrations based on formulation for the 4 samples

spike levels for exposure assessment

	intermediate solution conc. ug/ml	spiked volume ul	weight of spiked simulant g	spike conc mg/kg	Concentration ppb
BPA01	77.50	180	2031.5	**0.0069**	6.87
BPA02	77.50	550	1999.8	**0.0213**	21.31

spike level for enforcement purposes

	stock. solution conc ug/ml	spiked volume ul	weight of spiked simulant g	spike conc mg/kg	Concentration ppb
BPA03	775.0	200	2000.4	**0.0775**	77.48
BPA04	775.0	1500	2000.0	**0.581**	581.3

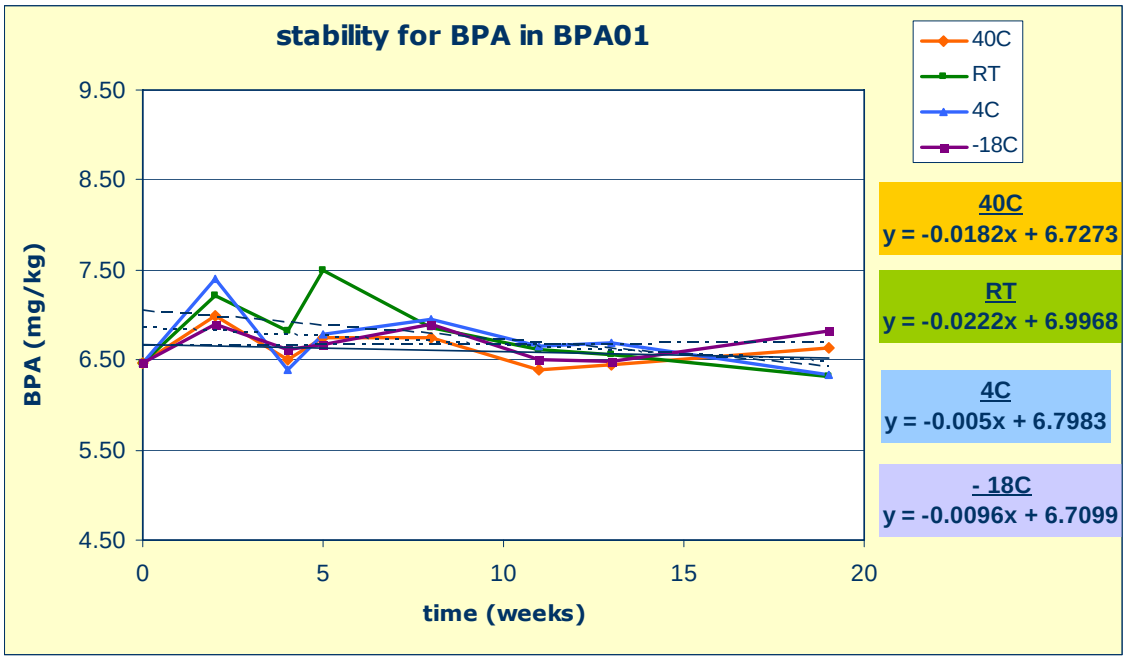
Annex 11: Results of the homogeneity study

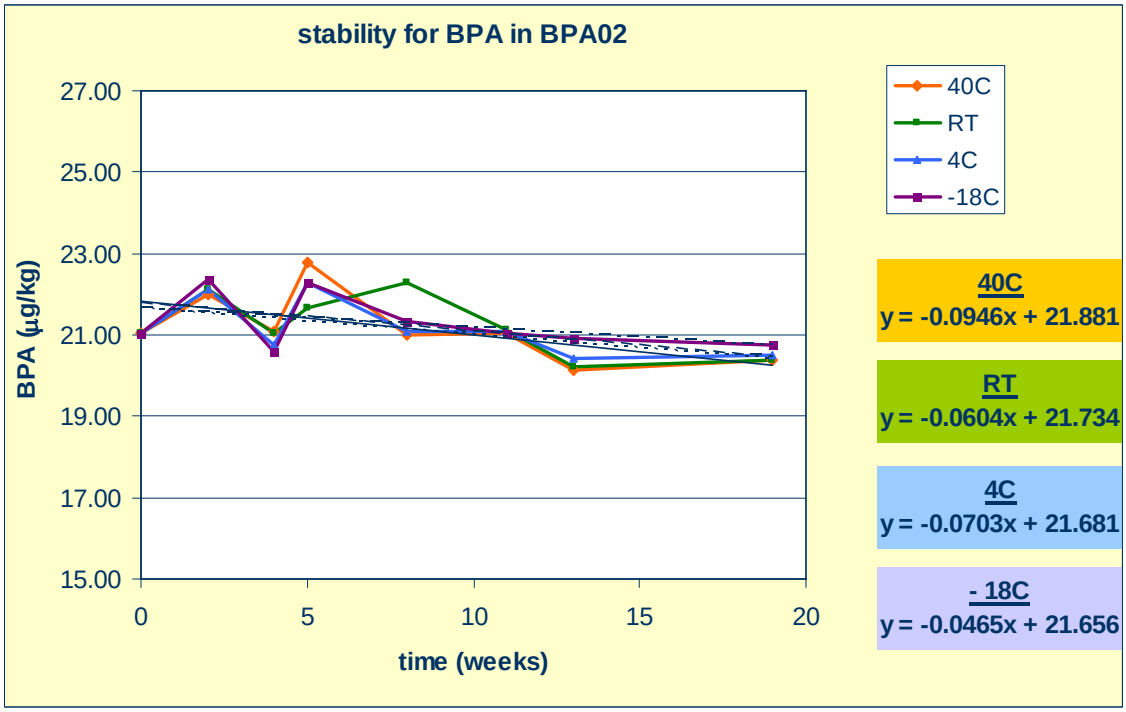
Table 9 Results from homogeneity test for BPA samples according to ISO 13528 and Harmonised protocol.

The test is performed using ProLab software against target standard deviation 1-3% which is much lower then the all potential target standard deviation that could be applied in the ILC02 (see table 6)

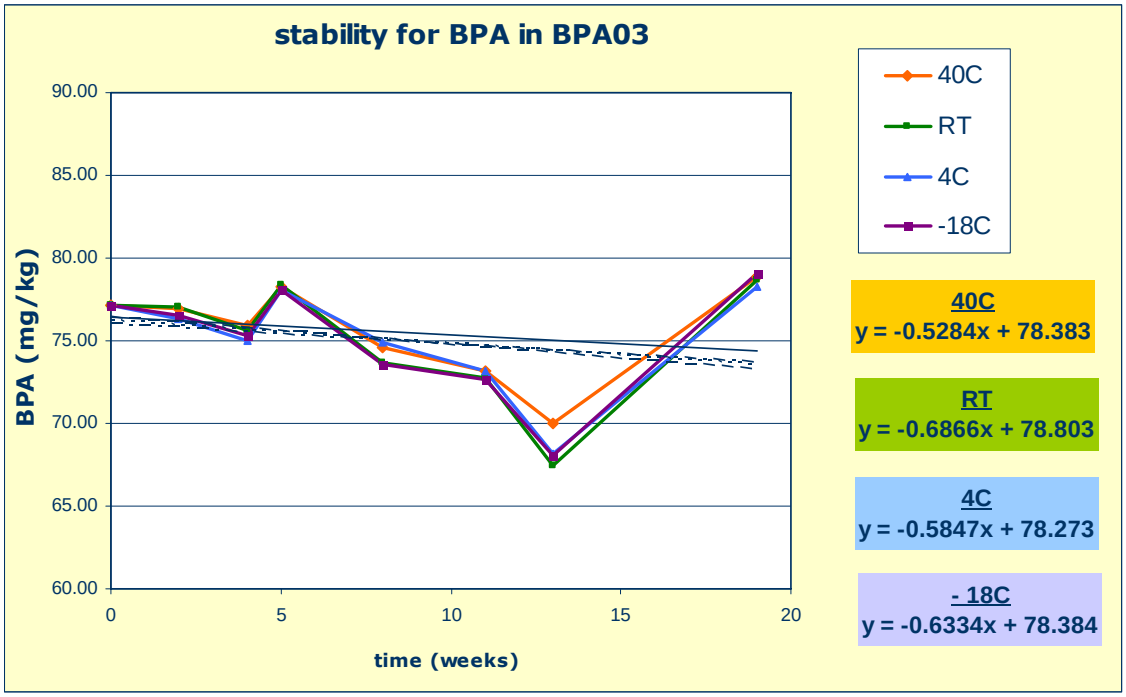
Sample	Measurand	Unit	Mean	s(analytical) %	s(samples) %	s(target) %	ISO 13528 Check for sufficient homogeneity	Harmonised Protocol - test on significant heterogeneity
BPA01	BPA	mg/kg	0.0061	2.21	0.846	3	OK	OK
BPA02	BPA	mg/kg	0.0196	0.498	0.489	2	OK	OK
BPA03	BPA	mg/kg	0.0715	0.301	0.221	1	OK	OK
BPA04	BPA	mg/kg	0.5333	0.262	0.000	1	OK	OK

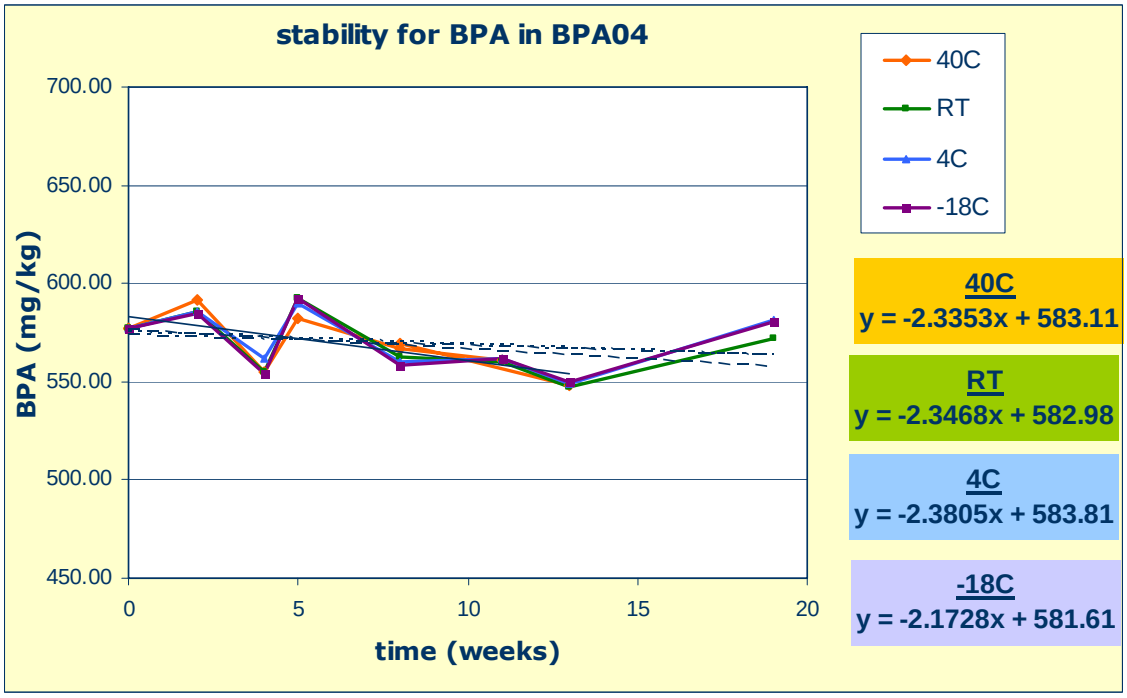
Annex 11 Results of the stability study





Annex 11 (continue) Results of the stability study





	reference value (mg/kg)	test condition	intercept (b0)	slope (b1)	s(b1)	t(α=0.95,g=5)·s(b1)
BPA01	6.87	**40°C**	6.681	**-0.0082**	0.0126	**0.0308**
		RT	7.046	**-0.0327**	0.0217	**0.0531**
		4°C	6.866	**-0.0196**	0.0210	**0.0514**
		-18°C	6.654	**0.0024**	0.0117	**0.0287**
BPA02	21.31	**40°C**	21.825	**-0.0826**	0.0441	**0.1079**
		RT	21.770	**-0.0680**	0.0396	**0.0969**
		4°C	21.652	**-0.0639**	0.0360	**0.0881**
		-18°C	21.657	**-0.0468**	0.0388	**0.0950**
BPA03	77.48	**40°C**	76.418	**-0.1053**	0.1850	**0.4527**
		RT	78.803	**-0.1661**	0.2867	**0.7016**
		4°C	76.203	**-0.1390**	0.2061	**0.5043**
		-18°C	76.046	**-0.1300**	0.2991	**0.7319**
BPA04	581.25	**40°C**	577.329	**-1.0913**	0.8171	**1.9994**
		RT	576.676	**-0.9901**	0.9207	**2.2528**
		4°C	575.736	**-0.6425**	0.9225	**2.2572**
		-18°C	574.024	**-0.5393**	0.9983	**2.4426**

European Commission

EUR 24550 EN – Joint Research Centre – Institute for Health and Consumer Protection
Title: Report of the interlaboratory comparison organised by the EU Reference for Laboratory Food Contact Material: ILC02 2009- Bisphenol A in 50% aqueous ethanol (milk simulant) - Laboratory performance and precision criteria of a harmonised method

Author(s): S. Bratinova, S. Valzacchi, M. Multari, S. Spalla and C. Simoneau

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Abstract

The Institute for Health and Consumer Protection (IHCP) of the European Commission's Directorate-General Joint Research Centre hosts the Community Reference Laboratory for Food Contact Materials (EURL-FCM). One of its core tasks is to organize interlaboratory comparisons (ILCs) among appointed National Reference Laboratories (NRLs).
This report presents the results of the second ILC exercise of the EURL-FCM for the year 2009, which focused on the determination of Bisphenol A (BPA) in 50% aqueous ethanol as a food simulant for milk. The general aim was to develop and perform the validation of a method for the analysis of Bisphenol A as model substance for a polycarbonate (PC), since it is a material that has typically been used as baby bottles and therefore typically in contact with mostly milk-type products. The strategy rose from the implementation of the new milk simulant 50% Ethanol (EtOH) in Commission Directive 2007/19/EC that current CEN standards for specific migration have not addressed yet.
The first specific intention of the exercise on BPA was to validate an extension of scope of EN13130 Part 13 to be extended to include this new simulant 50% EtOH, i.e. the method for the quantification of BPA in 50% EtOH in the range around the legislative specific migration limit (SML, 0.6 mg/kg). In addition, rather than just validating the method at levels close to the SML as required for compliance purposes, it was agreed that a second validation range would also be studied to allow validation data to be generated for exposure purposes. The test materials used in this exercise were spiked samples prepared by EURL-FCM with several level of Bisphenol A (Sigma Aldrich) in 50% ethanol. In total four 50% ethanol solutions containing different concentrations of Bisphenol A were provided for analysis encompassing concentrations of relevance to exposure determination and compliance determination. The homogeneity and stability studies were performed by the EURL-FCM laboratory. Standard operating procedures (SOPs) for the two approaches were also written. The spiked samples and SOP(s) were sent in August and the deadline for the submission of results was mid-October. Participation of local laboratories under NRLs was encouraged (by producing 60 samples). There were 31 participants from twenty-five countries to whom samples were dispatched and 26 of which submitted results. From the EURL-NRL network 18 laboratories out of 24 reported results. There were 3 guests from Spain and 3 from Germany that provided results as well. Participants were invited to report four replicates measurements in repeatability conditions. This was done by most of the participants. The ILC was closed permanently in the end of October for statistical interpretation. The results of analyses were received and statistically interpreted. The assigned values were obtained as a consensus values after applying the robust statistics to the results obtained from the participants. Laboratory results were rated with z-scores in accordance with ISO 13528 [1]. Standard deviations for ILC comparison (also called target standard deviations) were set based on Horwitz equation and Horrat ratio 0.5. The results and preliminary report were discussed in the plenary of December 2009. The participation of the laboratories was regarded as satisfactory for the aim of the precision experiment with regards of the numbers of received results. Absolute minimum of participating laboratories for conducting a precision experiment is 8. Some of the NRLs communicated the lack of the possibility to follow exactly the method for BPA determination as they used different analytical technique (e.g. LC-MS instead of HPLC/FLD). Since the aim of the ILC was directed towards method validation there was a communication to the participants that different techniques were not fit to the scope of this ILC. That was the reason for slightly lower percentage of reported test results as compared with the other ILC exercise of 2009. As a conclusion of the precision exercise on the quantification of Bisphenol A in the new milk simulant 50% ethanol, this ILC showed that:
The participation in the ILC was satisfactory for the purpose of the study towards validation with 24 laboratories.
The validation of the method based on HPLC-FLD according to the description based mostly on the previous CEN standard TS 13130-13 was successful with more than 8 valid results thanks to the proactive involvement of the NRLs.
The precision that can be suggested were of
15% reproducibility SD and 6% repeatability SD for the 0.0067 mg/kg level,
10% reproducibility SD and 4% repeatability SD for the 0.0.21 mg/kg level,
6% reproducibility SD and 2% repeatability SD for the 0.0.75 mg/kg level,
6% reproducibility SD and 0.2% repeatability SD for the 0.56 mg/kg level.

With respect to the scarcity of data previously available in the validation performed as reported in the CEN standard TS 13130-13 (issued version of 2005), this validation also provides a great breadth of valuable detailed and traceable raw data, which should prove extremely relevant for the creation of an extension of the standard from CEN.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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